Cell Biophysics II

Author: RNDr. Katarina Stroffekova, Ph.D.
Faculty of Science, Pavol Jozef Šafárik University in Košice

Email:katarina.stroffekova@upjs.sk

Content

COURSE DESCRIPTION:

- To teach basic principles and mechanisms underlying cell physiology and biophysics with the ***emphasis on molecular mechanisms*****.** Examples of mammalian diseases are used to illustrate key concepts.

COURSE OBJECTIVES

- 1. General knowledge of the principles of cellular function, including membrane, cytoplasmic and nuclear roles in normal and abnormal conditions.
- 2. In-depth understanding of the mechanisms underlying the cell functions at the molecular and subcellular level.
- 3. Awareness of cell pathologies that lead to specific disease symptoms and phenotypes.
- 4. The ability to apply learned principles to solve new problems (analysis & synthesis).

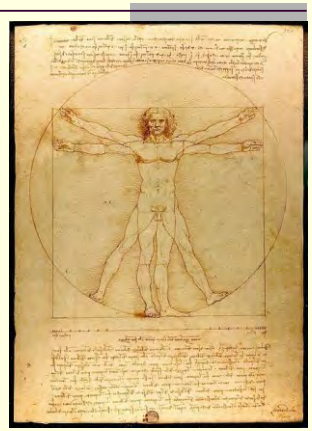
Recommended readings

- B. Alberts, A. Johnson, J. Lewis, M. Raff, K. Roberts, P. Walter: Molecular Biology of the Cell, 5th Edition, Garland Science 2008
- D.U. Silverthorn: Human Physiology – An Integrated Approach, Pearson/Benjamin Cummings 2010
- R.M.J. Cotterill: Biophysics – An Introduction, J.Wiley & Sons,Ltd. 2002
- G. Krauss: Biochemistry of Signal Transduction and Regulation, Wiley/VCH 2003
- M.B. Jackson: Molecular and Cellular Biophysics, Cambridge Univ. Press 2006
- D.G. Nicholls and S.J.Ferguson: Bioenergetics, Academic Press, 4th Ed. 2013

Historical overview of Physiology

Historical overview of Physiology

- **Physiology** (from Greek: φυσις, *physis*, “nature, origin”; and λόγος, *logos*, "speech" lit. "to talk about the nature (of things)" is the study of the physical, and chemical processes that take place during functions performed by living organisms.
- It is concerned with such basic activities as :
  - Reproduction
  - Growth
  - Metabolism
  - Excitation and contraction
- As they are carried out within the cells, tissues, organs, and organ systems of the body

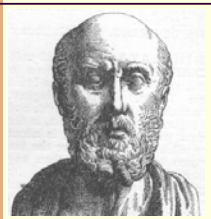


Leonardo DaVinci’s Vitruvian Man, an important early achievement in the study of **physiology**

Introduction to Physiology

- Physiology is intimately linked with anatomy and was historically considered a part of medicine. Its emphasis on investigating biological mechanisms with the tools of physics and chemistry made physiology a distinct discipline in the 19th century.
- Other major branches of scientific study that have grown out of physiology research include biochemistry, *biophysics*, paleobiology, biomechanics and pharmacology.

Introduction to Physiology



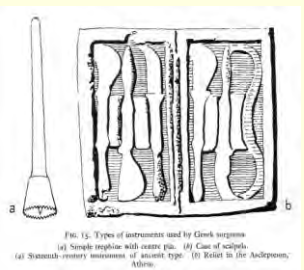


Fig. 15. Types of instruments used by Greek surgeons.
(a) Simple trephine with centre pin. (b) Case of scalpels.
(c) Sixteenth-century instruments of ancient type. (d) Relief in the Asclepieion, Athens.

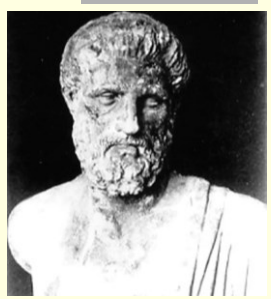
Adams, Francis (1891), *The Genuine Works of Hippocrates* New York: William Wood and Company.

- **The history of modern medicine starts with Hippocrates**
  - He was born around the year 460 BC on the Greek island of Kos (Cos) and is widely considered to be the **"Father of Western Medicine"**
  - **the first physician to reject superstitions** and beliefs that credited supernatural or divine forces with causing illness.
  - **separated the discipline of medicine from religion**, believing and arguing that disease was not a punishment inflicted by the gods, but rather the product of environmental factors, diet and living habits.
  - Hippocrates began to categorize illnesses as acute, chronic, endemic and epidemic.
  - He is given credit for the first description of the fingers clubbing an important diagnostic sign in chronic lung disease, lung cancer and cyanotic heart disease.
  - The first physician to accurately describe the symptoms of pneumonia, as well as epilepsy in children
  - Hippocrates was the first documented chest surgeon and his findings are still valid.

Garrison, Fielding H. (1966), *History of Medicine*, Philadelphia: W.B. Saunders Company.

Introduction to Physiology

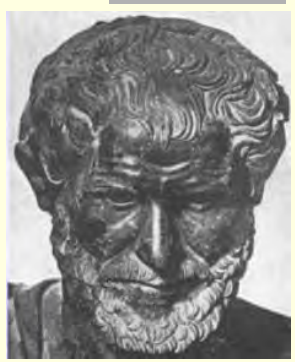
- The history of modern medicine continues with the two medical teachers Herophilus, whose treatise on anatomy may have been the first of its kind, and Erasistratus, regarded by some as the founder of physiology. Two of them are regarded as founders of the great medical school of Alexandria.
- **Herophilos (Herophilus)** (335-280 BC), was a Greek physician. He was born in Chalcedon in Asia Minor (now Kadiköy, Turkey).
  - **One of the founders of the scientific method**. He had introduced the experimental method to medicine, for he considered it essential to found knowledge on empirical bases.
  - **The first to base his conclusions on dissection of the human body**. He studied the brain, recognizing it as the center of the nervous and the site of intelligence. He also paid particular attention to the nervous system, distinguishing nerves from blood vessels and the motor from the sensory nerves. Other areas of his anatomical study include the eye, liver, pancreas, and the alimentary tract, as well as the salivary and genitalia. Amongst the latter, he is credited with the discovery of the ovum.



Garrison, Fielding H. (1966), *History of Medicine*, Philadelphia: W.B. Saunders Company.

Introduction to Physiology

- **Erasistratus of Chios** (304 BC- 250 BC) was a Greek anatomist and royal physician under Seleucus I Nicator of Syria.
  - considered atoms to be the essential body element, and he believed they were vitalized by external air (pneuma) that circulated through the nerves.
  - He then differentiated between the function of the sensory and motor nerves, and linked them to the brain.
  - Cardiovascular medicine was also greatly expanded due to Erasistratus' research. He concluded that the heart was not the center of sensations, but instead it functioned as a pump. He is credited for his description of the valves of the heart.
  - Erasistratus was among the first to distinguish between veins and arteries.
  - Some consider Erasistratus the first cardiac arrhythmologist, studying the rhythms of the heart.



Garrison, Fielding H. (1966), *History of Medicine*, Philadelphia: W.B. Saunders Company
Staden, Heinrich von (1989-04-20). *Herophilus: The Art of Medicine in Early Alexandria: Edition, Translation and Essays*. Cambridge University Press. p. 138. ISBN 9780521236461.

Galen

- Galen (c. AD 130-201), Greek physician.
- Galen's medical writings (comprising nearly a hundred works) became the standard source of medical knowledge for centuries.
- Pioneer in experimental work:
  - Demonstrated the function of the nervous system by cutting animals' spinal cords and different points and observing their resulting paralysis.
  - The first to consider the diagnostic value of taking a subject's pulse;
  - The first to identify several muscles.
- But Galen began from many faulty premises. His ***De usu partium* (*On the Use of Parts*)** depends on the teleological theories of Aristotle, and this led him to many inaccurate conclusions. His theories on the circulation of the blood, for instance, were not corrected until the sixteenth century and by the work of **William Harvey**.
  - Nutton V. From Galen to Alexander, aspects of medicine and medical practice in late antiquity. Dunbarton Oaks Papers. 38, 1984

Lithograph by Pierre Roche Vigneron. (Paris: Lith de Gregoire et Deneux, ca. 1865)

Middle Ages 200 – cca 1600 AD

- The taboo against desecrating the bodies of the dead goes back many centuries; it was prohibited by both ancient Greek and Roman religions.
- The taboo continued into the post-Classical era. Christian doctrine promises the resurrection of the body, which many thought to be impossible if a body were anatomized.

- Result – Research and development in physiology and medicine stagnated in Europe.

Middle Ages 200 – cca 1600 AD

- During the Middle Ages, the ancient Greek and Indian medical traditions were further developed by Muslim physicians.

- **Al-Razi (865-925 AD)** was a versatile Persian physician, philosopher, and scholar who made fundamental and enduring contributions to the fields of medicine, chemistry and philosophy (184 treatises).
  - As an **alchemist**, Razi is credited with the discovery of sulfuric acid, the "work horse" of modern chemistry. He also discovered ethanol and its refinement and use in medicine. He was unquestionably one of the greatest thinkers of the Islamic World, and had an enormous influence on European science and medicine.
  - As a **physician**, Razi made numerous advances in medicine through own observations and discoveries. He was an early proponent of experimental medicine and is considered the father of **pediatrics**. He was also a pioneer of **neurosurgery** and **opthalmology**.

- Al-Razi was a pure racionalist, extremely confident of the power of reason; he was widely regarded by his contemporaries and biographers as liberal and free from any kind of prejudice, very bold and daring in expressing his ideas without a qualm. He believed in man, progress and in "God the Wise".

- Iskandar, Albert (2006). "Al-Rāzī". *Encyclopaedia of the history of science, technology, and medicine in non-western cultures* (2nd ed.). Springer. pp. 155–156.

Middle Ages 200 – cca 1600 AD

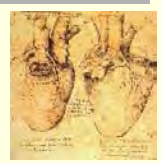
- **Avicenna** is the most known of Muslim physicians and continued work along the lines of Al-Razi.
- **Avicenna** (**Ibn Seena**) (980-1037), is regarded as a **father of early modern medicine and clinical pharmacology**. About 100 treatises were ascribed to Avicenna. The best-known amongst them, and that to which Ibn Sina owed his European reputation, is his 14-volume **The Canon of Medicine**, which was a standard medical text in Europe and the Islamic world up until the 18th century.
  - He introduced systematic experimentation and quantification into physiology
  - He was first to use experimental medicine, evidence-based medicine, clinical and randomized controlled trials, efficacy tests
  - Avicenna discovered the contagious nature of infectious diseases and introduced quarantine to limit the spread of contagious diseases. He asserts that **tuberculosis** was contagious, which was later disputed by Europeans, but turned out to be true.
  - He is a founder of clinical pharmacology, neuropsychiatry.
  - Avicenna introduced risk factor analysis, and the idea of a syndrome in the diagnosis of specific diseases – He described the symptoms and complications of **diabetes**.
  - He recognized the importance of dietetics and the influence of climate and environment on health.
  - He recognized the importance of hygiene.

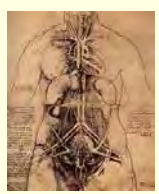
Avicenna (Persian philosopher and scientist) - Britannica Online Encyclopedia". Britannica.com. Retrieved 2012-01-07.

Middle Ages 200 – cca 1600 AD Europe

- The taboo against desecrating the bodies of the dead continued into the post-Classical era. Christian doctrine promises the resurrection of the body, which many thought to be impossible if a body were anatomized.
  - But physicians at Salerno's medical school defied Church authority, and **William of Saliceto** published a record of his dissections in ***Chirurgia*** in 1275.
- The **first public demonstration of human anatomy** came in **1315**; **Mondino de Luzzi**, an Italian surgeon, published ***Anatomia***, the first manual on dissection.
- The Renaissance saw a resurgence in interest in anatomy, in part urged by the studies of such artists as **Leonardo da Vinci**, who (in 1510) **demonstrated the homology of muscular structures in humans and animals** -- Leonardo, however, did not publish these drawings in his lifetime.

Leonardo da Vinci





Middle Ages 200 – cca 1600 AD Europe

- **Andreas Vesalius**, one of the founders of modern anatomy, received death sentence under the Inquisition for his dissections (1564). His experience, gained by performing dissections himself rather than relying on assistants, led him to question classical medical authorities. He discovered that their work was a pastiche of guesswork and analogy from animal anatomy. In 1543 he published his masterpiece, ***De corporis humani fabrica***, in seven volumes, providing the **first accurate drawings of human anatomy**.
- **Michael Servetus** (**Michel de Villeneuve**) (1511–53) was a Spanish physician who's certain religious beliefs views, particularly with regard to the Holy Trinity, that brought widespread condemnation from theologians both of the Reformation and of the Roman Catholic Church. He changed his name to **Michel de Villeneuve**, and spent some time in Paris studying medicine, where he became famous for his dissecting and medical abilities. He also discovered that some of the blood circulates through the lungs. **Servetus** was burned to death, in part for publishing his views against the doctrine of the Trinity. The execution was approved by Martin Luther, John Calvin, and Sir Thomas More.
- **Realdus Colombus** (1516-1559) also showed that the pulmonary veins carry blood, not air and **Hieronymus Fabricius ab Acquapendente** (1533-1619) described valves in veins, recognising them as general structures in the venous system and calling them little doors "ostiola".
- Dear, Peter. *Revolutionizing the Sciences: European Knowledge and Its Ambitions, 1500-1700*. Princeton: Princeton UP, 2001

Andreas Vesalius

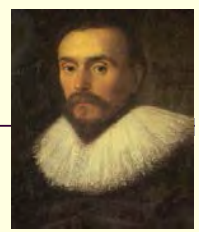
Modern Physiology starts in 17th century

- 1400+ years later after Galen

Renaissance 1600 - 1800

- Following from the Middle Ages, the Renaissance brought an increase of physiological research in the Europe that triggered the modern study of anatomy and physiology.
- The English physician **William Harvey** (1578-1657) finally elucidated the **system of blood circulation**.
  - Harvey showed experimentally the function of valves in maintaining centripetal flow in veins, thus establishing the true concept of a circulation propelled by the heart.
  - Harvey is considered "**father of modern physiology**".
  - Harvey also proposed the existence of capillaries, which would link arterial and venous systems, but was unable to demonstrate the capillary network due to the lack of a microscope.
  - Harvey based most of his conclusions on careful observations recorded during vivisections made of various animals during controlled experiments, being the first person to study biology quantitatively.

Kearney, Hugh (1971). *Science and Change 1500 – 1700*. New York: McGraw-Hill.
Butterfield, Herbert (1957). *The Origins of Modern Science* (revised ed.). New York: The Free Press.



William Harvey

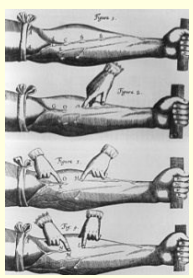


Illustration from:
Exercitatio anatomica

Modern Physiology starts in 17th century

- **Jan Baptista van Helmont** (1579-1644) was an Flemish chemist, physiologist and physician.
  - He developed concept of gases
  - Van Helmont wrote extensively on the subject of digestion. His own opinion was that digestion was aided by a chemical reagent, or "ferment", within the body, such as inside the stomach. In this way, van Helmont's idea was "very near to our modern concept of an enzyme."
  - He suggested that to undue acidity of the digestive juices, for example, was to be corrected by alkalines and vice versa;
  - He was thus a forerunner of **the iatrochemical school**, and did service to medicine by applying chemical methods to the preparation of drugs.
  - **Iatrochemists** – believed that physiology only involves chemical reactions
  - **Iatrophysicists** – believed that physiology only involves physical processes

Jan Baptista van Helmont

- **Giovanni Alfonso Borelli** (1608-1679) was a Renaissance Italian physiologist, physicist and mathematician.
  - Borelli's major scientific achievements are focused around his investigation into biomechanics. This originated in his studies of animal motion (***De Motu Animalium I*** ; ***De Motu Animalium II***). Borelli first suggested that the basis of muscle contraction lay in the muscle fibers. In particular, he compared the action of the heart to that of a piston. For this to work properly, he derived the idea that the arteries have to be elastic. For these discoveries, Borelli is labeled as **the father of modern biomechanics**.

Kearney, Hugh (1971). *Science and Change 1500 – 1700*. New York: McGraw-Hill.
Butterfield, Herbert (1957). *The Origins of Modern Science* (revised ed.). New York: The Free Press.

Modern Physiology starts in 17th century

- **Marcello Malpighi** (1628-1694) was an Italian physician.
  - Most of Malpighi's research results were published as articles in the journal of the Royal Society of England (founded 1660), first in 1661, in which he described anatomy of lung of a frog and **his discovery of capillaries**.
  - Malpighi used the compound microscope for studies on skin, kidney, and for the first interspecies comparison of the liver. He greatly extended the science of embryology and is regarded as **the founder of microscopic anatomy** and **the first histologist**.
- **Antonie Philips van Leeuwenhoek** (1632-1723) was a Dutch tradesman and scientist. He is commonly known as "**the Father of Microbiology**".
  - He is best known for his work on the improvement of the microscope and for his contributions towards microbiology. He was also the **first to record microscopic observations of muscle fibers, bacteria, spermatozoa and blood flow in capillaries**.

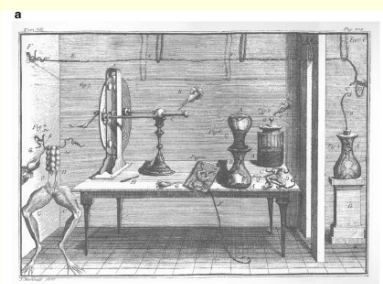
Kearney, Hugh (1971). *Science and Change 1500 – 1700.* New York: McGraw-Hill.

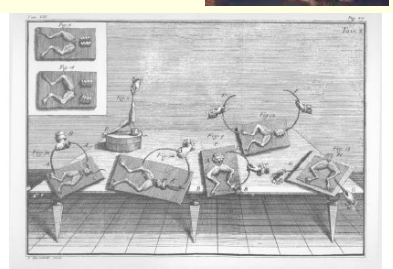
Butterfield, Herbert (1957). *The Origins of Modern Science* (revised ed.). New York: The Free Press.

Sorrenson, Richard (1996). "Towards a History of the Royal Society in the Eighteenth Century". *Notes and Records of the Royal Society of London* (The Royal Society) **50** (1): 29. doi:10.1098/rsnr.1996.0003

Physiology in 18th – 19th Century

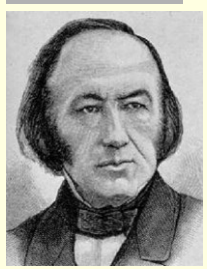
- **Luigi Galvani** (1737-1798) was an Italian physician and physicist. In 1771, he discovered that the muscles of dead frogs twitched when struck by a static electricity. He was a pioneer in modern obstetrics, and **discovered that muscle and nerve cells produce electricity**.





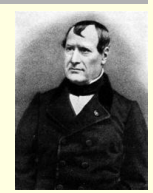
Physiology in 18th – 19th Century

- **Claude Bernard** (1813-1878) was a French physiologist. He is considered "one of the greatest of all men of science". One of the Bernard's classics is a book on scientific method, *An **Introduction to the Study of Experimental Medicine*** (originally published in 1865). He is considered as the **"Father of Physiology"**.
  - Bernard investigated:
    - carbohydrate metabolism in humans
    - ANS and described many of his functions
  - His **greatest contribution** was his **statement of the principle that living organisms are never at rest but constantly undergo dynamic changes to maintain equilibrium** "HOMEOSTASIS".



Physiology in 18th – 19th Century

- **Marie Jean Pierre Flourens** (1794-1867) was a French physiologist, the founder of experimental brain science and a pioneer in anesthesia.
  - Flourens pioneered the experimental method of carrying out localized lesions of the brain in living rabbits and pigeons and carefully observing their effects on control of muscle movement and strength, sensibility and behavior.
  - **He was able to demonstrate convincingly for the first time that the main divisions of the brain were indeed responsible for largely different functions**. The experiments led Flourens to the conclusion that the cerebral hemispheres are responsible for higher cognitive functions, that the cerebellum regulates and integrates movements, and that the medulla controls vital functions, such as circulation, respiration and general bodily stability.



- **Johannes Peter Müller** (1801-1858), was a German physiologist, and comparative anatomist not only known for his discoveries but also for his ability to synthesize knowledge.
  - Müller made contributions in numerous domains of physiology, in particular increasing understanding of the voice, speech and hearing, as well as the chemical and physical properties of lymph, chyle, and blood.
  - **He demonstrated that perceptions were determined only by sensory organ that received sensory input**.

Physiology in 20 th Century

- Among the most important advances of the 20th century are:
  - The discovery of new hormones
  - Recognition of the role of vitamins
  - Development of techniques to record activity of heart and brain
  - Greater understanding of metabolism, the role of enzymes, and the immune system
  - The role of neurotransmitters in transmission of nerve impulses

Physiology in 20th and 21st Century

1900	Karl Landsteiner discovers the A, B, and O blood groups.
1904	Ivan Pavlov wins the Nobel Prize for his work on the physiology of digestion.
1910	Sir Henry Dale describes properties of histamine.
1918	Earnest Starling describes how the force of the heart's contraction relates to the amount of blood in it.
1921	John Langley describes the functions of the autonomic nervous system.
1923	Sir Frederick Banting, Charles Best, and John Macleod win the Nobel Prize for the discovery of insulin.
1932	Sir Charles Sherrington and Lord Edgar Adrian win the Nobel Prize for discoveries related to the functions of neurons.
1936	Sir Henry Dale and Otto Loewi win the Nobel Prize for discovery of acetylcholine in synaptic transmission.
1939–47	Albert von Szent-Georgi explains the role of ATP and contributes to the understanding of actin and myosin in muscle contraction.
1949	Hans Selye discovers the common physiological responses to stress.
1953	Sir Hans Krebs wins the Nobel Prize for his discovery of the citric acid cycle.
1954	Hugh Huxley, Jean Hanson, R. Niedergerde, and Andrew Huxley propose the sliding filament theory of muscle contraction.
1962	Francis Crick, James Watson, and Maurice Wilkins win the Nobel Prize for determining the structure of DNA.
1963	Sir John Eccles, Sir Alan Hodgkin, and Sir Andrew Huxley win the Nobel Prize for their discoveries relating to the nerve impulse.
1971	Earl Sutherland wins the Nobel Prize for his discovery of the mechanism of hormone action.
1977	Roger Guillemin and Andrew Schally win the Nobel Prize for discoveries of the peptide hormone production by the brain.
1981	Roger Sperry wins the Nobel Prize for his discoveries regarding the specializations of the right and left cerebral hemispheres.
1986	Stanley Cohen and Rita Levi-Montalcini win the Nobel Prize for their discoveries of growth factors regulating the nervous system.
1994	Alfred Gilman and Martin Rodbell win the Nobel Prize for their discovery of the functions of G-proteins in signal transduction in cells.
1998	Robert Furchgott, Louis Ignarro, and Ferid Murad win the Nobel Prize for discovering the role of nitric oxide as a signaling molecule in the cardiovascular system.
2004	Linda B. Buck and Richard Axel win the Nobel Prize for their discoveries of odorant receptors and the organization of the olfactory system.

The most important findings – 2 citation per decade

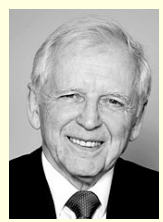
Physiology in 21st Century

- Americans Andrew Z. Fire and Craig C. Mello won the Nobel Prize in medicine (2006) for discovering a powerful way to turn off the effect of specific genes, opening a potential new avenue for fighting diseases as diverse as cancer and AIDS.

- **Mario R. Capecchi**, **Martin J. Evans** and **Oliver Smithies** won the Nobel Prize in medicine (2007) for their discoveries of **"principles for introducing specific gene modifications in mice by the use of embryonic stem cells"**

The Nobel Prize in Physiology or Medicine 2008

"for his discovery of human papilloma viruses causing cervical cancer"
"for their discovery of human immunodeficiency virus"



Harald zur Hausen	Françoise Barré-Sinoussi	Luc Montagnier
1/2 of the prize	1/4 of the prize	1/4 of the prize
Germany	France	France
German Cancer Research Centre Heidelberg, Germany	Regulation of Retroviral Infections Unit, Virology Department, Institut Pasteur Paris, France	World Foundation for AIDS Research and Prevention Paris, France
b. 1936	b. 1947	b. 1932

The Nobel Prize in Physiology or Medicine 2009

"for the discovery of how chromosomes are protected by telomeres and the enzyme telomerase"

Elizabeth H. Blackburn

1/3 of the prize

USA

University of California
San Francisco, CA, USA

b. 1948
(in Hobart, Tasmania, Australia)

Carol W. Greider

1/3 of the prize

USA

Johns Hopkins University School of Medicine
Baltimore, MD, USA

b. 1961

Jack W. Szostak

1/3 of the prize

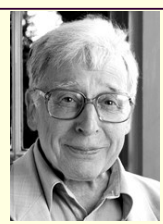
USA

Harvard Medical School;
Massachusetts General Hospital
Boston, MA, USA;
Howard Hughes Medical Institute

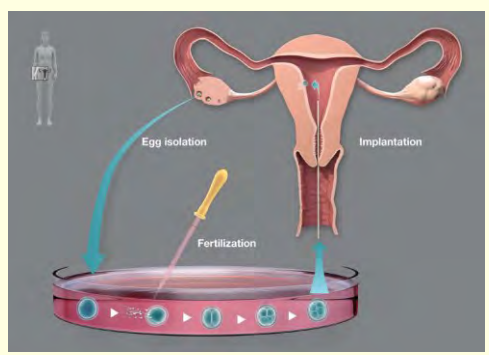
b. 1952
(in London, United Kingdom)

The Nobel Prize in Physiology or Medicine 2010

"for the development of in vitro fertilization"

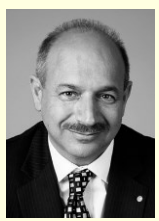


Robert G. Edwards
University of Cambridge,
Cambridge, United Kingdom



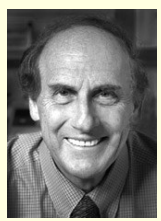
The Nobel Prize in Physiology or Medicine 2011 *was awarded one half jointly to Bruce A. Beutler and Jules A. Hoffmann and the other half to Ralph M. Steinman*

- *"for their discoveries concerning the activation of innate immunity"* and *"for his discovery of the dendritic cell and its role in adaptive immunity"*.



Bruce A. Beutler

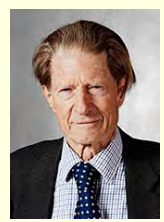
Jules A. Hoffmann



Ralph M. Steinman

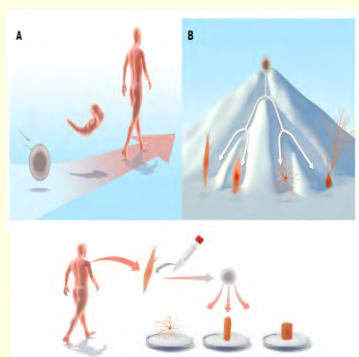
The Nobel Prize in Physiology or Medicine 2012 *was awarded jointly to Sir John B. Gurdon and Shinya Yamanaka*

- *"for the discovery that mature cells can be reprogrammed to become pluripotent"*



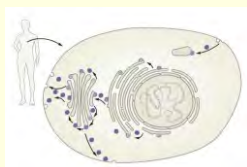
Sir John B. Gurdon

Shinya Yamanaka



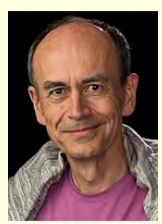
The Nobel Prize in Physiology or Medicine 2013 *was awarded jointly to James E. Rothman, Randy W. Schekman and Thomas C. Südhof.*

- ***"for their discoveries of machinery regulating vesicle traffic, a major transport system in our cells".***



James E. Rothman

Randy W. Schekman



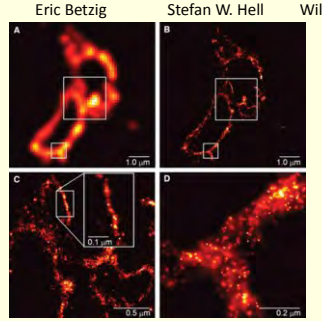
Thomas C. Südhof

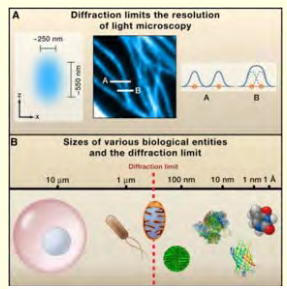
Nobel Prize in Chemistry 2014 Eric Betzig, Stefan W. Hell and William E. Moerner *„ for the development of super-resolved fluorescence microscopy ".*

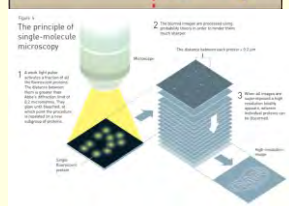
Eric Betzig

Stefan W. Hell

William E. Moerner







Nobel Prize in Chemistry 2015 Eric Betzig, Stefan W. Hell and William E. Moerner *„ for mechanistic studies of DNA repair"*.

Tomas Lindahl Paul Modrich Aziz Sancar

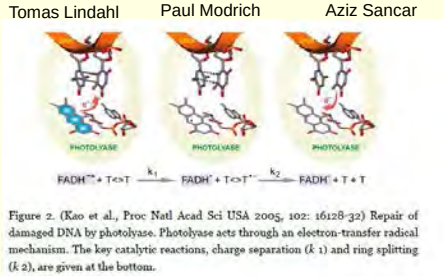


Figure 2. (Kao et al., Proc Natl Acad Sci USA 2005, 102: 16128-32) Repair of damaged DNA by photolyase. Photolyase acts through an electron-transfer radical mechanism. The key catalytic reactions, charge separation (k 1) and ring splitting (k 2), are given at the bottom.

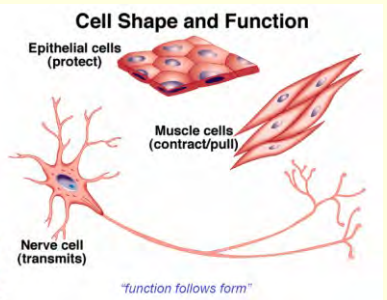
Introduction to Cell Biophysics

Introduction to Physiology

- Anatomy deals with the structure (morphology) of the body parts and their relationships to one another
- Physiology studies the function of the body's structural machinery
  - **Anatomists** rely on **observations**
  - **Physiologists** use **experimentation**

Physiology

- The functional role of a part depends on how it is constructed – *Principle of complementarity*
- Physiology: study of how body works to maintain life
- Pathophysiology: how physiological processes are altered in disease or injury



Cell Biophysics: study of how cells work

Experimentation

Scientific Method

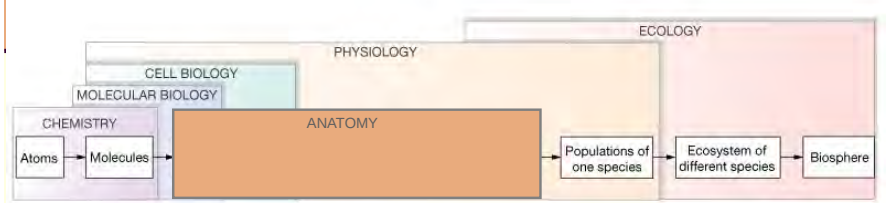
- 1. Form a testable hypothesis about observations
- 2. Conduct and analyze experiments to test hypothesis
- 3. Draw conclusions about whether or not results support hypothesis
- 4. Develop a **theory**
  - General statement explaining natural phenomena that is based on proven hypotheses

Testing of Hypotheses

- Involves:
  - Experimental and control groups
  - Quantitative measurements performed blindly
  - Analysis of data using statistics
  - Modeling

Levels of organization and the related fields of study

- Physiology defined:
  - **Function** explains why certain event takes place
  - **Mechanism** explains how it is done
- Organization of life
  - The cell is the unit of life
  - Cells, tissues, organs, organ systems & organisms

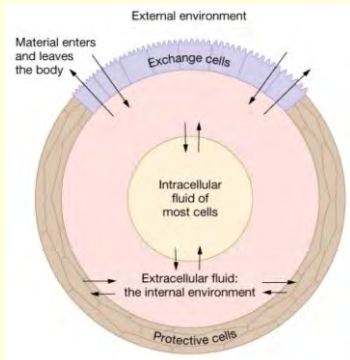


Physiology

- Considers the operation of specific organ systems
  - Renal – kidney function
  - Neurophysiology – workings of the nervous system
  - Cardiovascular – operation of the heart and blood vessels
- Focuses on the functions of the body, often at the *cellular* or *molecular* level
- Understanding physiology also requires a knowledge of physics (*biophysics*), which explains electrical currents, blood pressure, and the way muscle uses bone for movement etc.

Necessary Life Functions

- **Maintaining boundaries** – the internal environment remains distinct from the external
  - Cellular level – accomplished by plasma membranes
  - Organismal level – accomplished by the skin
- **Movement** – locomotion, propulsion (peristalsis), and contractility
- **Responsiveness** – ability to sense changes in the environment and respond to them
- **Digestion** – breakdown of ingested foods



Adopted from Silverthorn 2010

Necessary Life Functions

- **Metabolism** – all the chemical reactions that occur in the body (cell)
- **Excretion** – removal of wastes from the body (cell)
- **Reproduction** – cellular and organismal levels
  - Cellular – an original cell divides and produces two identical daughter cells
  - Organismal – sperm and egg unite to make a whole new person
- **Growth** – increase in size of a body part or of the organism

Necessary Life Functions

- Fundamental characteristics of life are those traits shared by all organisms
- The traits include:

movement responsiveness growth reproduction
respiration digestion absorption
excretion circulation assimilation

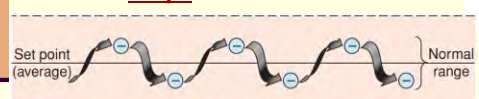
These traits constitute METABOLISM

Survival Needs – Factors needed to be present to sustain life in operating limits

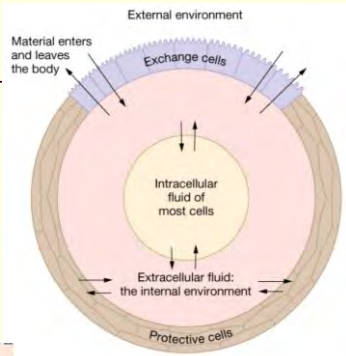
- **Nutrients** – chemical substances used for energy and cell building
- **Oxygen** – needed for metabolic reactions
- **Water** – provides the necessary environment for chemical reactions
- **Maintaining normal body temperature** – necessary for chemical reactions to occur at life-sustaining rates
- **Atmospheric pressure** – required for proper breathing and gas exchange in the lungs

Homeostasis

- Is maintenance of a state of dynamic constancy
  - In which conditions are stabilized above and below a physiological set point
    - By **negative feedback loops**



- As much as 90% of the body energy resources goes to maintaining homeostasis

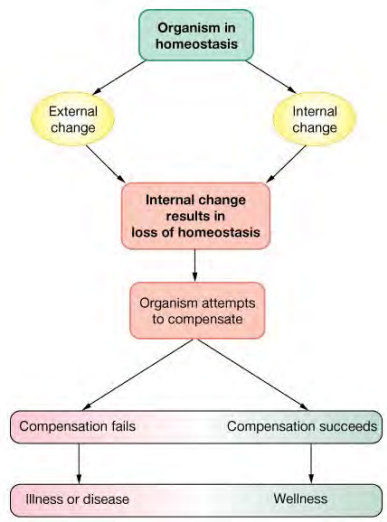


Adopted from Silverthorn 2010

Claude Bernard: **"the principle that living organisms are never at rest but constantly undergo dynamic changes to maintain equilibrium „**

Homeostasis

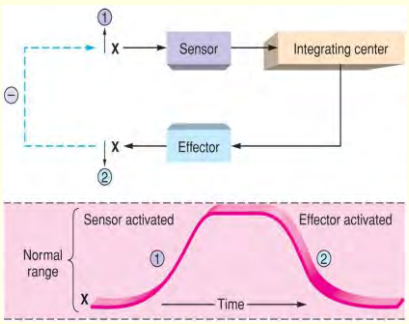
- Most medical treatments and therapies are design to return one to a condition of homeostasis
- Successful compensation
  - Homeostasis reestablished
- Failure to compensate
  - Pathophysiology
    - Illness
    - Death



Adopted from Silverthorn 2010

Negative Feedback Loops

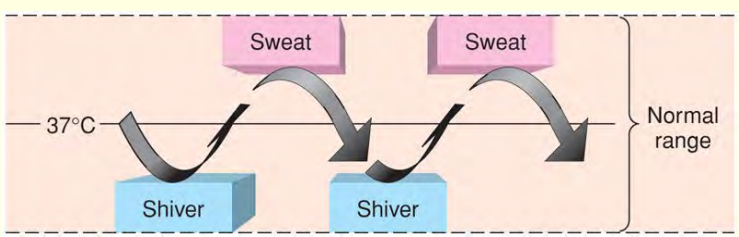
- **Sensor**:
  Detects deviation from **set point**
- **Integrating center**:
  Determines response
- **Effector**:
  Produces response



Adopted from Silverthorn 2010

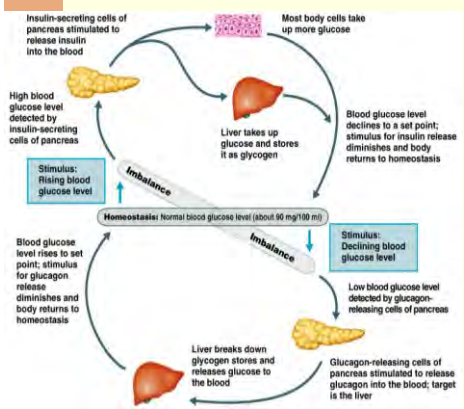
Homeostasis Regulatory Mechanisms

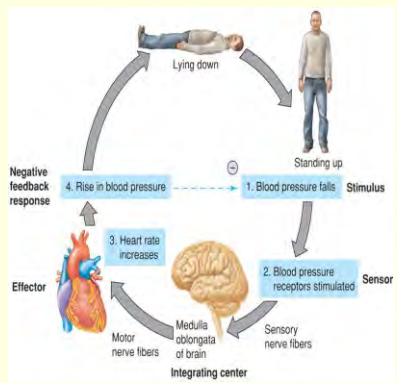
- **Intrinsic** control is built into organ/cell being regulated
  - Example: kidney maintain Na^+ level within itself
    cell maintain resting membrane potential
- **Extrinsic** control comes from outside of organ/ cell
  - Example:
    - Nerves innervate target tissue to enhance or inhibit function
    - Body temperature is controlled by antagonistic effects of sweating and shivering
    - Insulin control blood glucose levels by regulating ***cell*** glucose uptake



Adopted from Silverthorn 2010

Negative Feedback



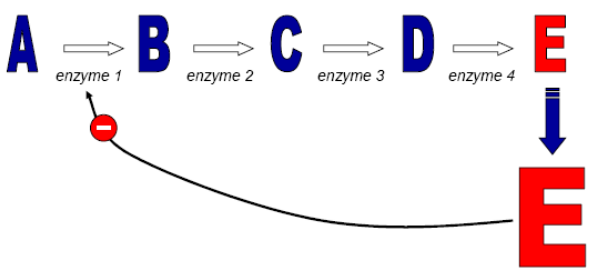


Adopted from Silverthorn D.U.: Human Physiology – An Integrated Approach, Pearson/Benjamin Cummings 2010

Negative Feedback

Negative Feedback in a Metabolic Pathway

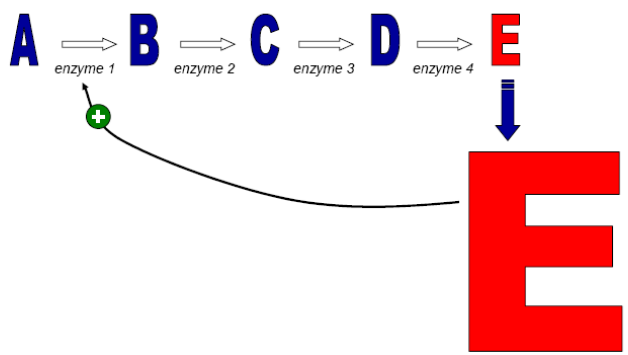
Goal of pathway: make product "E" from starting material "A"



Positive Feedback

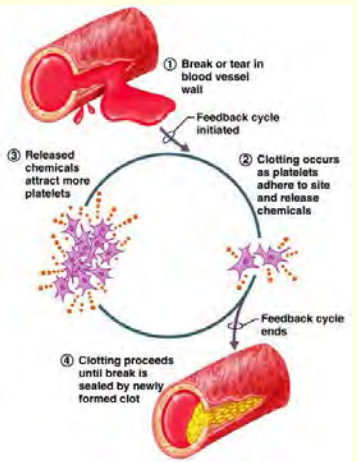
Why aren't positive feedback loops commonly found?

Positive feedback systems lead to further INSTABILITY



Positive Feedback

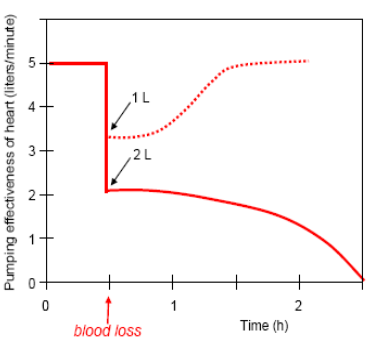
- is rare because it amplifies changes
- In positive feedback systems, the output enhances or exaggerates the original stimulus
- Example:
  - Regulation of blood clotting
  - the LH surge that causes ovulation
  - Positive feedback between the uterus and oxytocin secretion occurs during labor

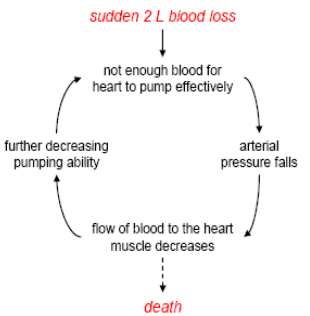


Adopted from Silverthorn 2010

Positive Feedback

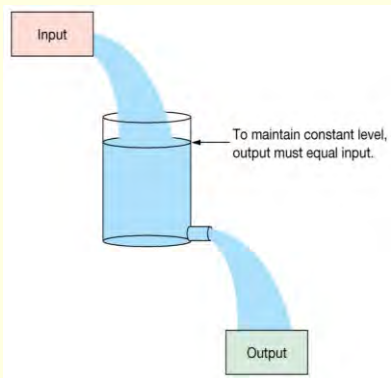
Death by positive feedback





Themes in Physiology/Cell Biophysics

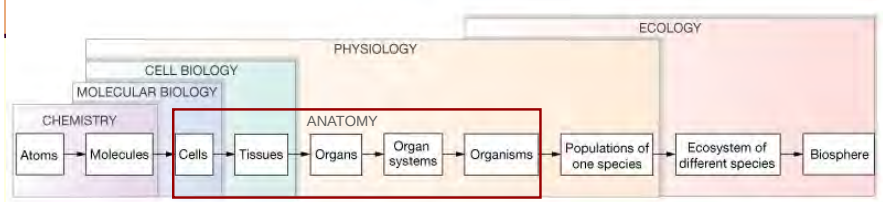
- Homeostasis
- Structure/function relationships
- Integration of systems
- Communication
- Membranes & exchange
- Energy
- Mass balance
- Mass flow & resistance



Adopted from Silverthorn 2010

Levels of organization of human body

- The cell is the ***smallest*** unit of life
- Cells, tissues, organs, organ systems & organism (human body)
  - **Organs** are anatomical and functional units made of two or more primary tissues
  - **Systems** are groups of organs working together to maintain homeostasis

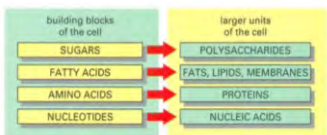


Approximate Chemical Composition of a Cell

TABLE 2–4 Approximate Chemical Compositions of a Typical Bacterium and a Typical Mammalian Cell

COMPONENT	PERCENT OF TOTAL CELL WEIGHT: *E. COLI* BACTERIUM	PERCENT OF TOTAL CELL WEIGHT: MAMMALIAN CELL
H_2O	70	70
Inorganic ions (Na^+, K^+, Mg^{2+}, Ca^{2+}, Cl^-, etc.)	1	1
Miscellaneous small metabolites	3	3
Proteins	15	18
RNA	6	1.1
DNA	1	0.25
Phospholipids	2	3
Other lipids	–	2
Polysaccharides	2	2
Total cell volume	2×10^{-12} cm^3	4×10^{-9} cm^3
Relative cell volume	1	2000

Proteins, polysaccharides, DNA, and RNA are macromolecules. Lipids are not generally classed as macromolecules even though they share some of their features; for example, most are synthesized as linear polymers of a smaller molecule (the acetyl group on acetyl CoA), and they self-assemble into larger structures (membranes). Note that water and protein comprise most of the mass of both mammalian and bacterial cells.



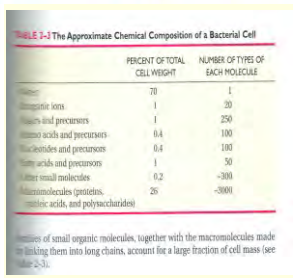


TABLE 2-1 The Approximate Chemical Composition of a Bacterial Cell

	PERCENT OF TOTAL CELL WEIGHT	NUMBER OF TYPES OF EACH MOLECULE
	70	1
	1	20
	1	250
	0.4	100
	0.4	100
	1	50
	0.2	~300
	26	~3000

Adopted from Alberts et al 2008

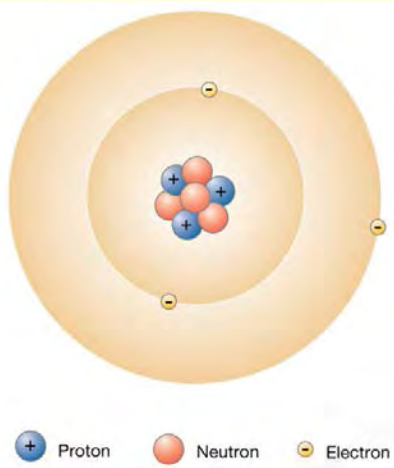
Chemical components of cell

Biomolecules

- Make up of atoms, ions, & molecules
- Bonds combine atoms, form molecules
- Concentrations
- Biomolecules

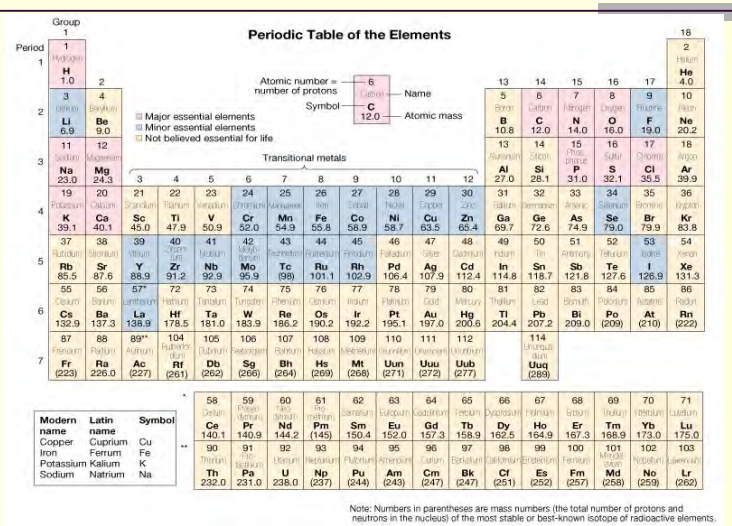
Atoms and Elements

- Structure of an atom
  - Protons
  - Electrons
  - Neutrons
- Mass
- Charge
- Nucleus
- Electron orbitals



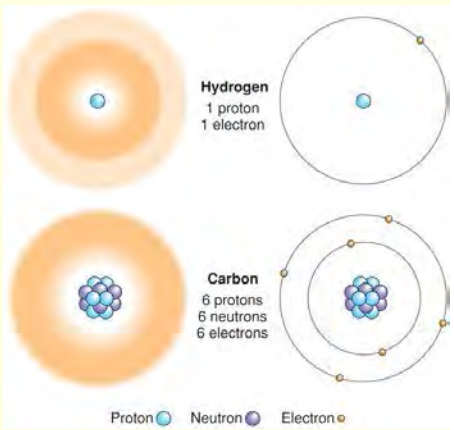
Adopted from Silverthorn 2010

All the Elements



Atoms continued

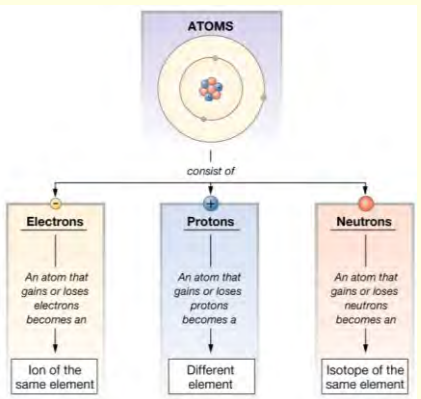
- **Atomic mass** is sum of protons and neutrons in an atom
- **Atomic number** is number of protons in an atom
- **Electron shells** or **orbitals** are in layers around nucleus
  - Number of shells depends on atomic number
  - First shell can contain only 2 electrons
  - Second shell can contain up to 8 electrons
  - Electrons in more distant shells have higher energy
- **Valence electrons** are those in outermost shell
  - These can participate in chemical reactions and form bonds



Adopted from Silverthorn 2010

Ions and Isotopes

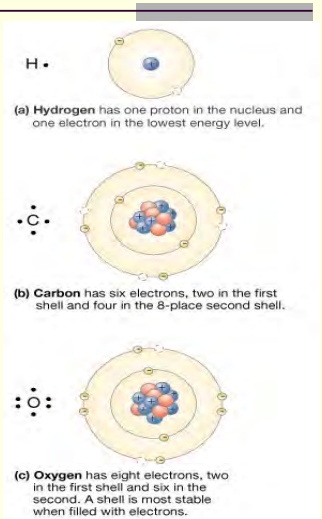
- Ions have charge
  - Cations +
  - Anions -
- Isotopes vary mass
  - Neutrons
  - Radioisotopes
    - Unstable nuclei
    - Emit energy - radiation
    - Medical uses as tracers



Adopted from Silverthorn 2010

Molecules and Compounds

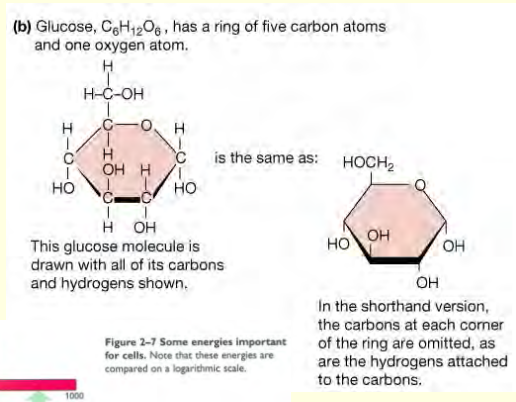
- The most common elements in biosystems
  - Carbon (C)
  - Oxygen (O)
  - Hydrogen (H)



Adopted from Silverthorn 2010

Molecules and Compounds

- Bonds capture energy
  - Bonds link atoms
- Molecules
  - Molecular weight
  - Chemical formula



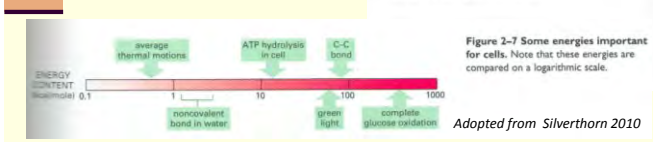
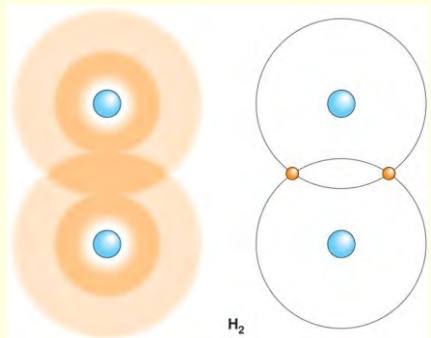


Figure 2–7 Some energies important for cells. Note that these energies are compared on a logarithmic scale.

Adopted from Silverthorn 2010

Types of Chemical Bonds

- **Covalent bonds**
  - Common in biosystems
  - Share a pair of electrons

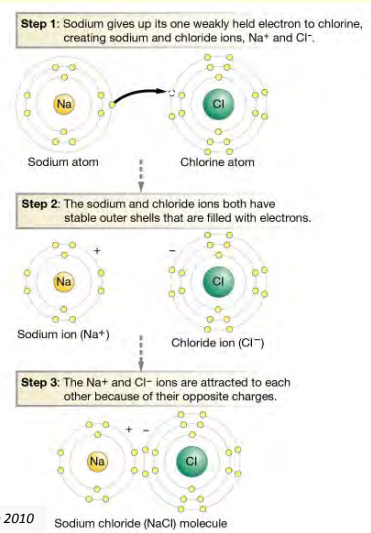


Adopted from Silverthorn 2010

Types of Chemical Bonds

- Ionic Bonds
  - Transfer an electron
  - Opposite charges attract

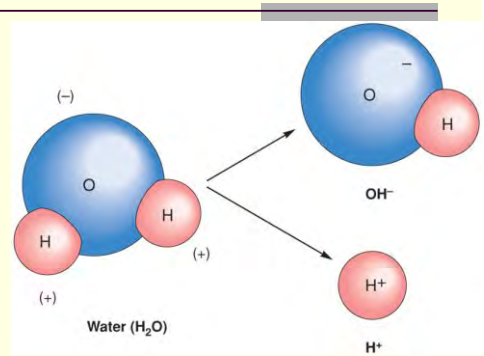
Important Ions of the Body			
Cations		**Anions**	
Na^+	Sodium	Cl-	Chloride
K^+	Potassium	HCO_3^-	Bicarbonate
Ca^{2+}	Calcium	HPO_4^-	Phosphate
H^+	Hydrogen	SO_4^{2-}	Sulfate
Mg^{2+}	Magnesium		

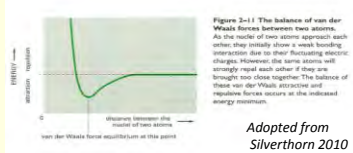


Adopted from Silverthorn 2010

Types of Chemical Bonds

- **Hydrogen bonds**
- In polar bonds electrons are shared unequally
  - Pulled more toward one atom
  - Have + and – poles
  - Oxygen, nitrogen, phosphorous have strong pull
    - Tend to form polar molecules - H_20
  - Weak partial bonds
  - Water surface tension
- **Van der Waals forces** – weaker then hydrogen bonds

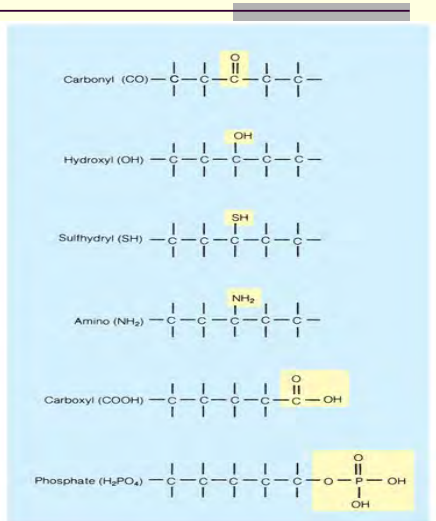




Adopted from Silverthorn 2010

Functional Groups

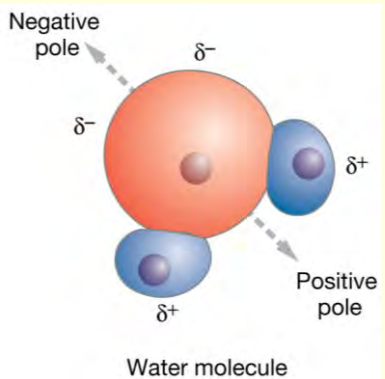
- Direct reactivity of a molecule
- Common examples in biosystems:
  - Carbonyl group forms ketones and aldehydes
  - Hydroxyl group forms alcohols
  - Carboxyl group forms organic acids (lactic and acetic acids)



Adopted from Silverthorn 2010

Types of Chemical Bonds

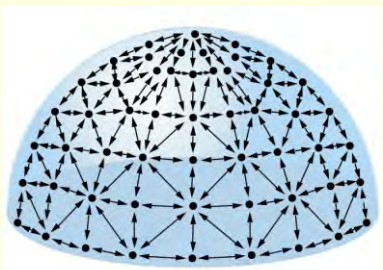
- Polarity of Molecules
  - Partial charges on regions of molecule
  - Soluble in polar solvents (i. e. H_2O)
- Non polar molecules
  - No regional partial charges
  - Do not dissolve easily in water (i.e. lipids)

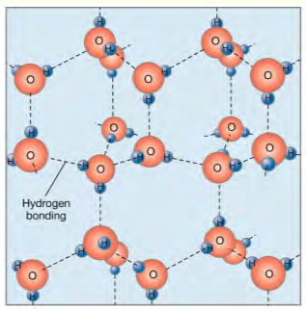


Adopted from Silverthorn 2010

Hydrogen Bonds (H-bonds)

- Strong polarity
- Attracts to self
- Surface tension
  - Form droplets
  - Thin films

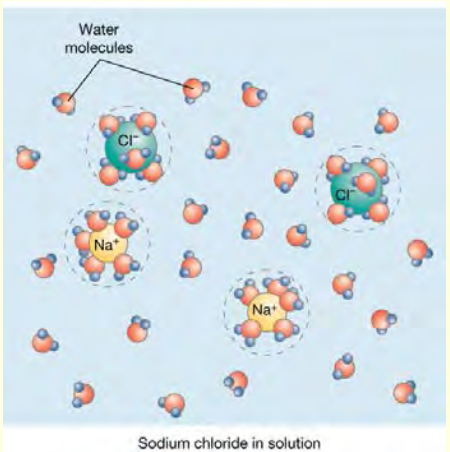




Adopted from Silverthorn 2010

Solutions: Water is the main Solvent in Biosystems

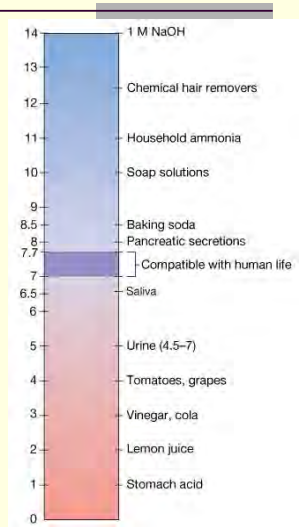
- Solutes dissolve in liquids
- Solvents dissolve solutes
- Solution: solute dissolves in solvent
- Solubility, ease of dissolving
  - Hydrophobic
  - Hydrophilic



Sodium chloride in solution

Hydrogen Ion Concentration (pH) in Biosystems

- Acid - contributes H^+ to solution
  $CO_2 + H_2O$ <=> H_2CO_3 <=> $H^+ + HCO_3^-$
- Base - decreases H^+ in solution
  ($NH_3 + H_2O$ <=> NH_4^+ OH^-)
- Buffer minimizes changes of pH



Hydrogen Ion Concentration (pH) in Biosystems

Common Acids and Bases			
Acid	Symbol	Base	Symbol
Hydrochloric acid	HCl	Sodium hydroxide	NaOH
Phosphoric acid	H_3PO_4	Potassium hydroxide	KOH
Nitric acid	HNO_3	Calcium hydroxide	$CA(OH)_2$
Sulfuric acid	H_2SO_4	Amonium hydroxide	NH_4OH
Carbonic acid	H_2CO_3		

- Blood pH
- Normal range of pH is 7.35 – 7.45
  - Maintained by buffering action
  - Acidosis occurs if pH < 7.35
  - Alkalosis occurs if pH > 7.45

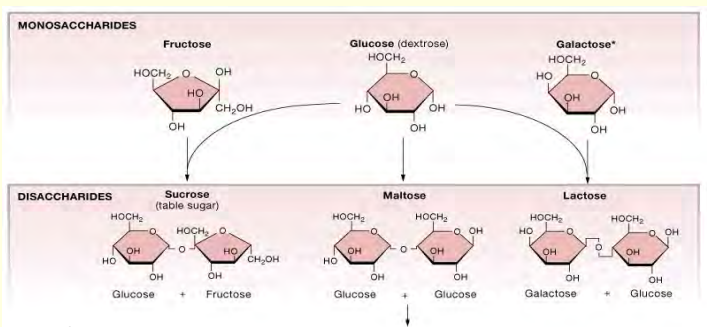
Biomolecules – synthesized by living organisms and contain C (carbon) atoms.

- Carbohydrates
- Lipids
- Proteins
- Nucleotides

Carbohydrate Biomolecules: Carbon, Hydrogen & Oxygen

$C_n(H_2O)_n = (CH_2O)_n$

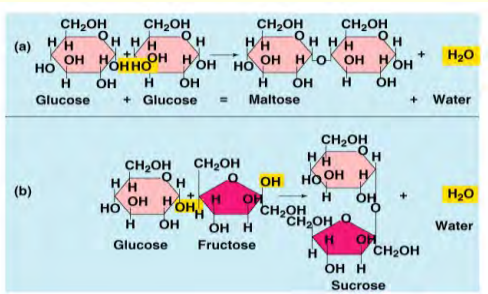
- Complex carbohydrates: polymers (polysaccharides)
- "Simple sugars" monosaccharides (glucose, ribose)



Adopted from Silverthorn 2010

Formation of Disaccharides

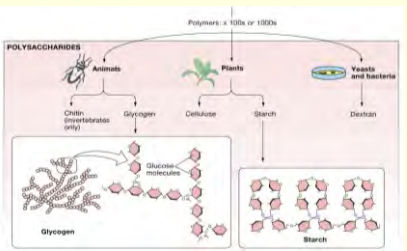
- **Dehydration synthesis:**
  - 2 monosaccharides are covalently bonded together, producing maltose or sucrose.
    - An H^+ and OH^- removed, producing H_20.
  - Requires specific enzymes.

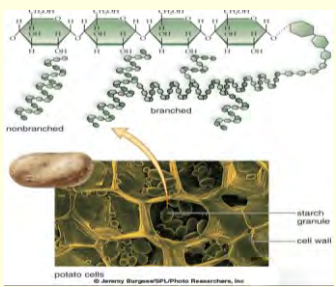


Adopted from Alberts et al. 2008

Carbohydrate Biomolecules

- Polysaccharides are made of many carbon rings
- **Polysaccharide**:
  - Numerous monosaccharides joined covalently.
    - Starch (thousands of glucose joined), glycogen (repeating glucose joined that are highly branched).
  - Mechanism for storing energy with less osmotic H_20 movement.
- **Glycogen** is the storage form in animals
- **Starch** is the storage form in plants

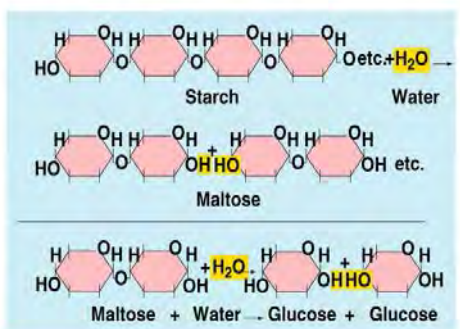




Adopted from Silverthorn 2010

Hydrolysis of Polysaccharides

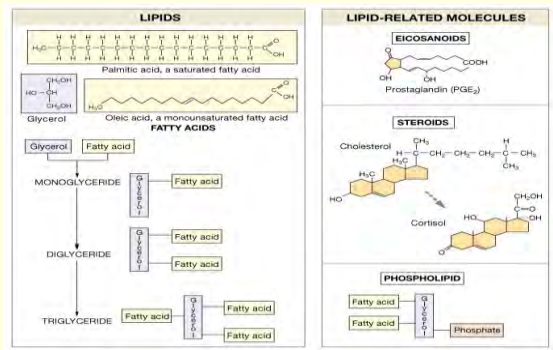
- **Digestion reaction**
  - Reverse of dehydration synthesis.
- Polysaccharide hydrolyzed into disaccharides, then to monosaccharides.
  - H_2O molecule split, H^+ added to one monosaccharide subunit, OH^- to the other.



Adopted from Alberts et al. 2008

Lipids

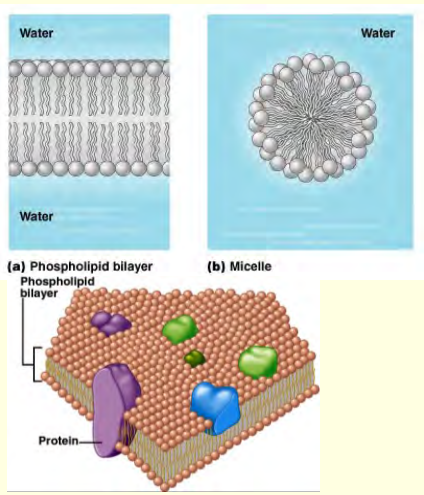
- Composed of primarily hydrogen and carbon atoms –
  - non-polar covalent bonds; hydrophobic
- Triglycerides: Glycerol,Fatty acid chains
- Eicosanoids, Steroids & Phospholipids



Adopted from Silverthorn 2010

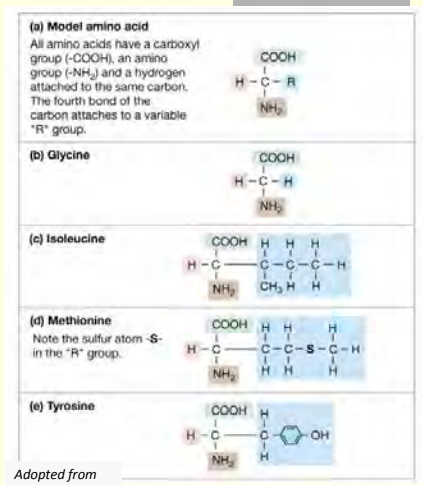
Lipids - Phospholipids

- Phospholipids aggregate into micelles in water
  - Polar part interacts with water; nonpolar part is hidden in middle
- Act as surfactants by reducing surface tension



Proteins: Amino acid polymers

- Amino Acids (20 total): 9 essential, amino group, acid group
- Protein structure: polypeptides, primary -quaternary



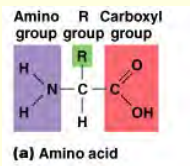
Adopted from Silverthorn 2010

Essential Amino Acids

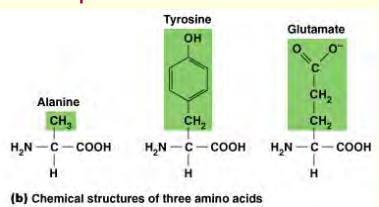
Name	Recommended daily intake in human adults (mg per Kg body weight) according WHO	for 70Kg human (mg)
P Phenylalanine	14 (sum with Tyrosine)	980
V Valine	10	700
T Tryptophan	3	245
T Threonine	7	490
I Isoleucine	10	700
M Methionine	13 (sum with Cysteine)	910
H Histidine	unknown, 28 in infants (? sum with arginine)	(? 1960)
A Arginine	unknown, required for infants, maybe seniors	(?)
L Lysine	12	840
L Leucine	14	980

Proteins: Amino acid polymers

Basic structure



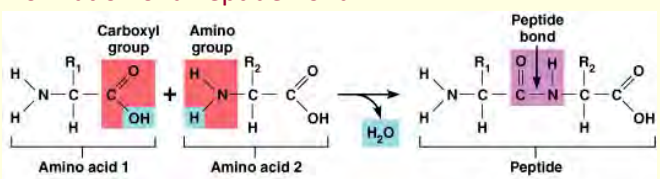
Examples of amino acids



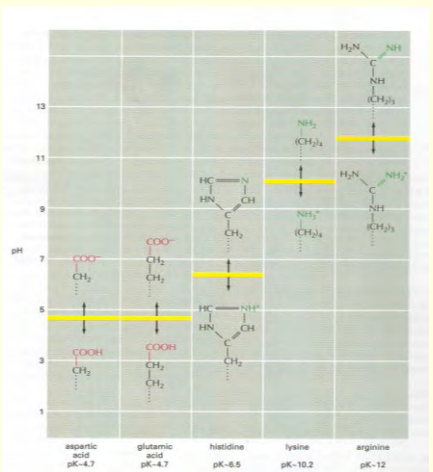
1-9 AA - oligopeptides
10-100 AA - polypeptides
> 100 AA - proteins

Formation of a Peptide Bond

Adopted from Alberts et al. 2008



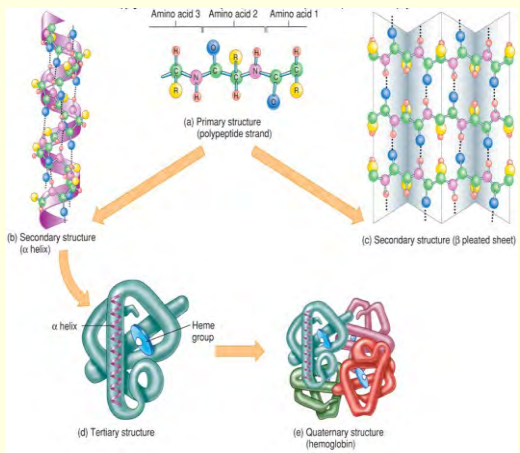
Overall charge on Amino Acid



Adopted from Silverthorn 2010

Proteins - Structure

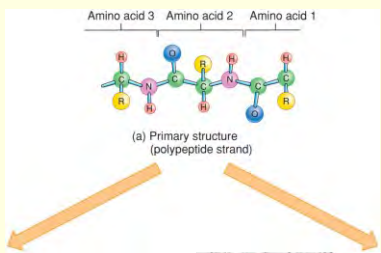
- Can be described at four levels

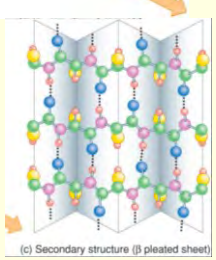


Adopted from Alberts et al. 2008

Proteins - Structure

- Primary structure is its sequence of amino acids
- Secondary structure is caused by weak H bonding of amino acids
  - Results in alpha helix or beta pleated sheet

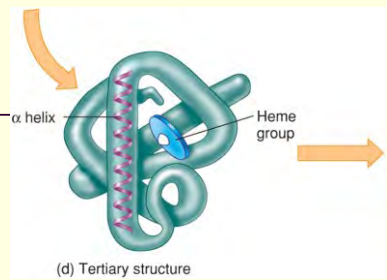


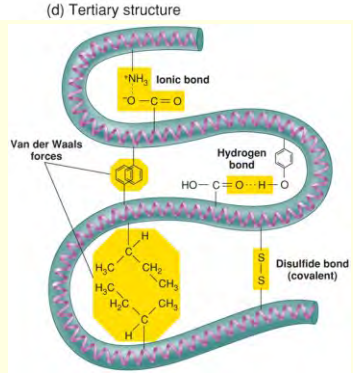


Adopted from Alberts et al. 2008

Protein - Structure

- Tertiary structure is caused by bending and folding of polypeptide chains to produce 3-dimensional shape
  - Formed and stabilized by weak bonds between functional groups
    - H or van der Waals
    - Ionic
    - disulfide
  - Not very stable; can be denatured by heat, pH

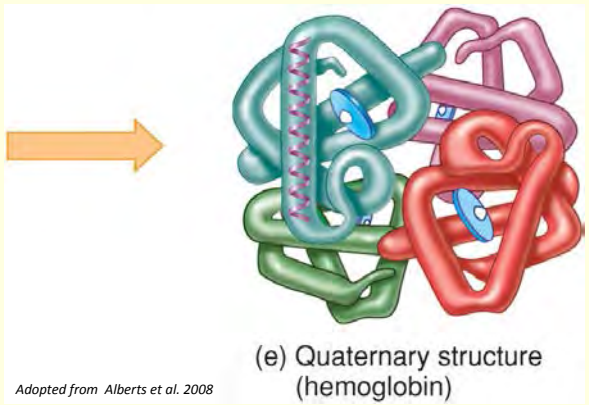




Adopted from Alberts et al. 2008

Protein - Structure

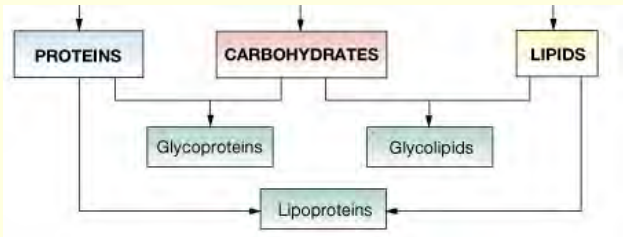
- Quaternary structure forms when a number of polypeptide chains are covalently joined



Adopted from Alberts et al. 2008

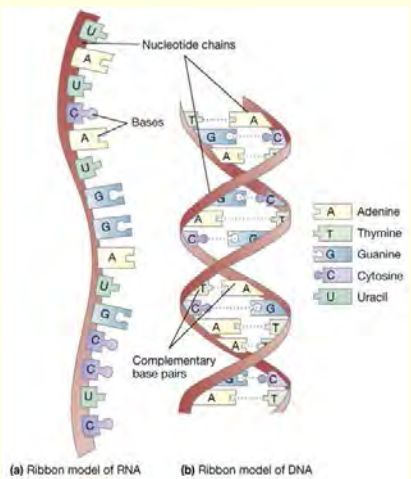
Combination Biomolecules

- Lipoproteins (blood transport molecules)
- Glycoproteins (membrane structure)
- Glycolipids (membrane receptors)



Nucleotides, DNA and RNA

- Composition
  - Base
  - Sugar
  - Phosphate
- Transmit and store
  - Information (genetic code)
  - Energy transfer molecules
    - ATP
    - Cyclic AMP
    - NAD & FAD



Adopted from Alberts et al. 2008

Nucleotides

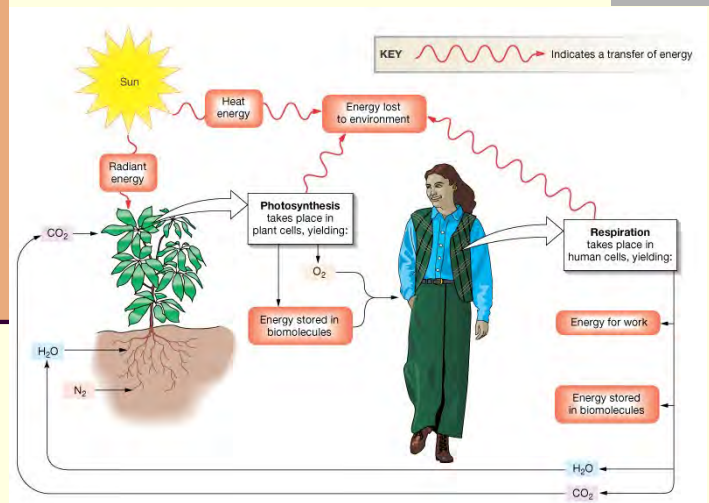
- Phosphate group(s)
- 5-carbon carbohydrate
  - Ribose
  - Deoxyribose
- Base containing carbon-nitrogen ring
  - pyrimidines (cytosine, thymine, uracil)
  - purines (adenine, guanine)

Adopted from Alberts et al. 2008

Cell metabolism and bioenergetics

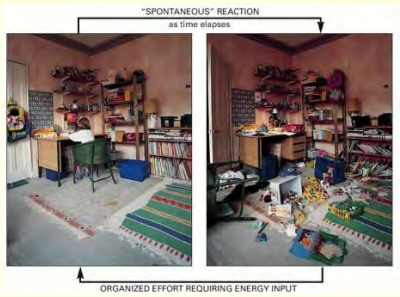
Energy (E) Transfer Overview

Energy cycling between enviroment and living organisms – fundamental concept in Biology



Bioenergetics

- Flow of energy in living systems obeys:
- 1st law of thermodynamics:
  - Energy can be transformed, but it cannot be created or destroyed.
- 2nd law of thermodynamics:
  - Energy transformations increase entropy (degree of disorganization of a system).
  - Only free energy (energy in organized state) can be used to do work.
    - Systems tend to go from states of higher free energy to states of lower free energy.



Adopted from Alberts et al. 2008

What is the purpose of energy use in living systems?

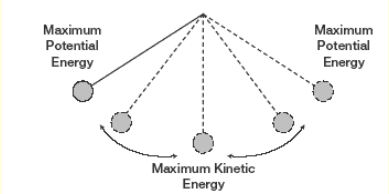
- 1. Chemical work – the forming or breaking chemical bonds
- 2. Transport – movement of nutrients and waste (concentration gradients)
- Mechanical work – movement of cells or organizms, organelles, changing shape etc.

Forms of Energy

All forms of energy fall under two categories

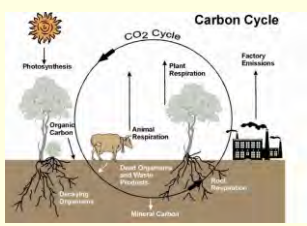
POTENTIAL
Stored energy or energy of position (gravitational)

KINETIC
Energy of motion (motion of waves, electrons, atoms, molecules, and substances)



The change of potential energy into kinetic energy, and kinetic energy into potential energy, in a pendulum.

Cells (living systems) obtain energy from the organic molecules => *Carbon cycle*



Cell Metabolism

- A. enzymes
- B. redox reactions
- C. cellular respiration

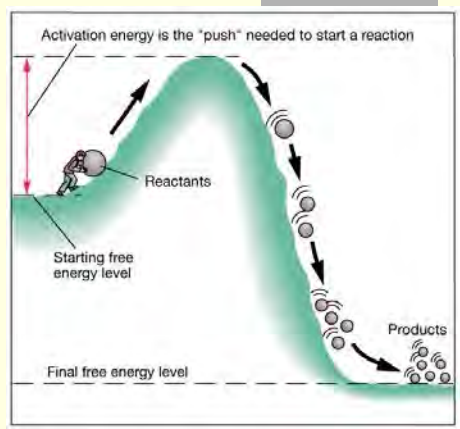
1. carbohydrate pathways
   - a. glycolysis (aerobic & anaerobic)
   - b. gluconeogenesis
   - c. oxidative phosphorylation
   - d. glycogenesis/glycogenolysis
2. lipid pathways
3. protein pathways

Cell Metabolism

- Cell metabolism includes all the chemical reactions that occur in a cell.
- Cell metabolism is driven by ***enzymes***.
- All metabolic reactions involve energy transformations. Detailed description of cell metabolism is a subject of *Biochemistry*.
- Metabolic reactions are divided into 2 categories:
  - **Catabolic:**
    - Release energy.
    - Breakdown larger organic molecules into smaller molecules.
    - Serve as primary sources of energy for synthesis of ATP.
    - Example – cellular respiration
  - **Anabolic or biosynthetic**:
    - Require input of energy.
    - Synthesis of large energy-storage molecules.
    - Example:
      - Amino acids => proteins
      - Carbohydrates => glycogen

Energy and Chemical Reactions

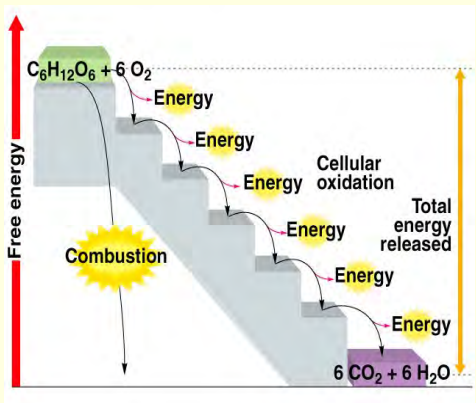
- Energy transfer
- Activation energy
- Endergonic
- Exergonic
- Net free energy



Adopted from Silverthorn 2010

Endergonic and Exergonic Reactions

- Endergonic (catabolic):
  - Chemical reactions that require an input of energy to make reaction "go."
    - Products must contain more free energy than reactants.
- Exergonic (anabolic):
  - Convert molecules with more free energy to molecules with less.
  - Release energy in the form of heat.
    - Heat is measured in calories.



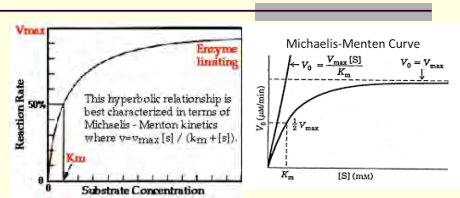
Adopted from Silverthorn 2010

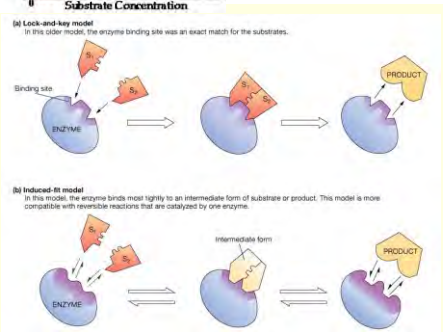
Enzymes and Coenzymes

- Subclass of proteins
- Increase rate of chemical reactions.
- Most enzymes are proteins with diverse structure.
- Functionally are biological catalysts:
  - Chemicals that increases the rate of a reaction.
  - Are not changed at the end of the reaction.
  - Do not change the nature of the reaction or final result.
  - Lower the activation energy required.
    - Amount of energy required for a reaction to proceed.

Mechanism of Enzyme Action

- Enzymes are protein catalysts, they influence the kinetics but **not the thermodynamics** of a reaction
  - Increase the rate of a chemical reaction
  - Do not alter the equilibrium
- Type of enzymatic reactions:
  - Oxidation–reduction
  - Hydrolysis–dehydration
  - Addition–subtraction exchange
  - Ligation
- Rate of enzyme-catalyzed reactions measured by the rate substrates are converted to products.

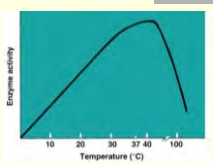


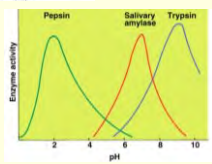


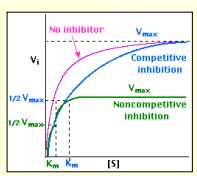
Adopted from Silverthorn 2010

Control of Enzyme Activity

- Factors influencing rate:
  - Temperature.
  - pH.
  - concentration of cofactors and coenzyme.
  - concentration of enzyme and substrate.
  - Stimulatory and inhibitory effects of products of enzyme action.

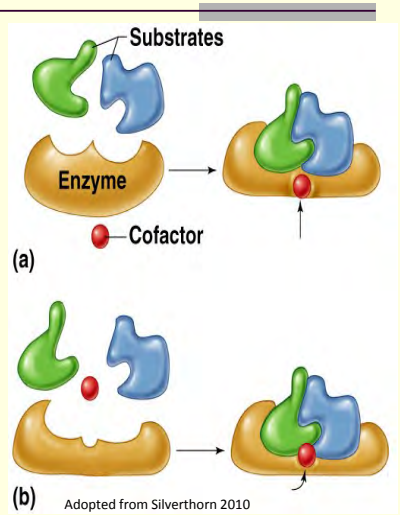






Cofactors and Coenzymes

- Needed for the activity of specific enzymes.
- Cofactor:
  - Attachment of cofactor causes a conformational change of active site.
  - Participate in temporary bonds between enzyme and substrate.
- Coenzymes:
  - Organic molecules derived from H_20 soluble vitamins.
  - Transport H^+ and small molecules from one enzyme to another.



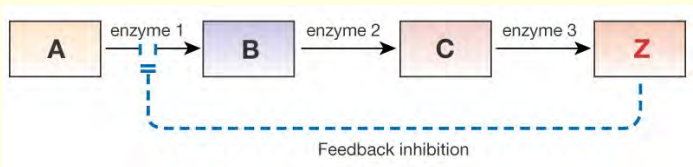
Adopted from Silverthorn 2010

Reversible Reactions

- Some enzymatic reactions are reversible.
  - Both forward and backward reactions are catalyzed by same enzyme.
- $H_20 + C0_2 \overset{ca}{\rightleftharpoons} H_2C0_3$
- Law of mass action:
  - Principal that reversible reactions will be driven from the side of the equation where concentration is higher to side where concentration is lower.

Control of Metabolic Pathways

- Feedback inhibition
- Enzyme modulators
- No enzyme
- Enzyme isolation
- Energy availability - ATP



Diagnostically Important Enzymes

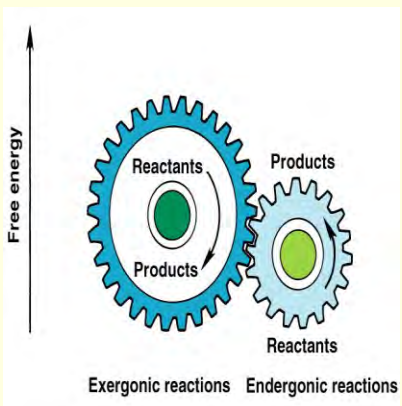
Enzyme	Related disease
Acid phosphatase	Prostate cancer
Alkaline phosphatase	Bone and liver diseases
Amylase	Pancreatic disease
Creatine kinase	Heart and muscle diseases
Glutamate dehydrogenase	Liver disease
Lactate dehydrogenase	Tissue damage to heart, skeletal muscle, liver and red blood cells

Cell Metabolism = Metabolism of Biomolecules

- A. enzymes
- B. redox reactions
- C. cellular respiration
- 1. carbohydrate pathways
  - a. glycolysis (aerobic & anaerobic)
  - b. gluconeogenesis
  - c. oxidative phosphorylation
  - d. glycogenesis/glycogenolysis
- 2. lipid pathways
- 3. protein pathways

Coupled Reactions: ATP

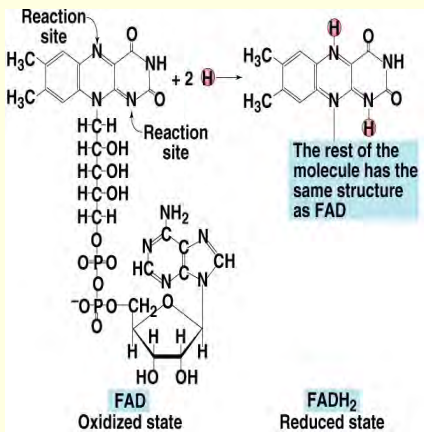
- Cells must maintain highly organized, low-entropy state at the expense of free energy.
  - Cells cannot use heat for energy.
- Energy released in exergonic reactions used to drive endergonic reactions.
  - Require energy released in exergonic reactions (ATP) to be directly transferred to chemical-bond energy in the products of endergonic reactions.



Adopted from Silverthorn 2010

Oxidation-Reduction

- Reduced:
  - Molecule/atom gains electrons.
- Reducing agent:
  - Molecule/atom that donates electrons.
- Oxidized:
  - Molecule/atom loses electrons.
- Oxidizing agent:
  - Molecule/atom that accepts electrons.
- ***Reduction and oxidation are always coupled reactions.***
- May involve the transfer of H^+ rather than free electrons.
- Molecules that serve important roles in the transfer of H^+ are **NAD** and **FAD**.
  - Coenzymes that function as hydrogen carriers.

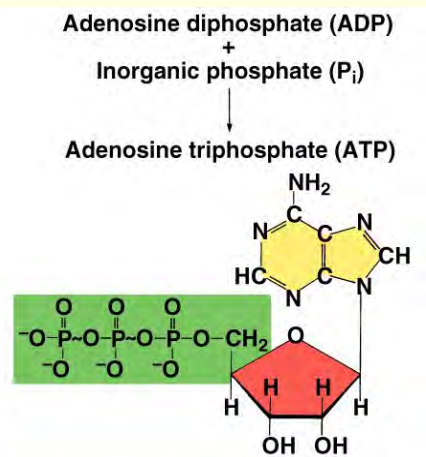


Adopted from Alberts et al 2008

Formation of ATP

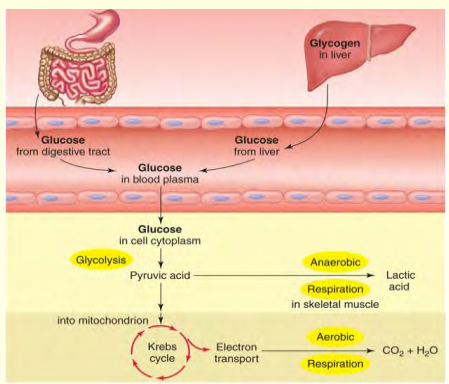
- Formation of ATP requires the input of a large amount of energy.
  - Energy must be conserved, the bond produced by joining P_i to ADP must contain a part of this energy.
  - This energy released when ATP converted to ADP and P_i.
- ATP can be made 2 ways:
  - Direct (substrate-level) phosphorylation
    - Where ATP is generated when bonds break
    - Both ATPs in glycolysis are made this way
    - 2 ATPs/glucose in Krebs are made this way
  - Oxidative phosphorylation in Krebs
    - Where ATP generated by ETC
    - 30-32 ATPs are made this way

- **ATP is the universal energy carrier in the cell**.



Cellular Respiration = Glucose Oxidation

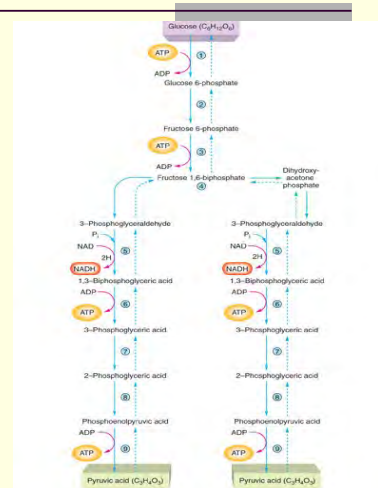
- Glucose breakdown requires three steps:
  - 1. Glycolysis
  - 2. Krebs or Citric Acid Cycle
  - 3. Oxidative Phosphorylation - Electron Transport System
- Altogether, the breakdown of one glucose molecule results in 36 ATP molecules.



Adopted from Silverthorn 2010

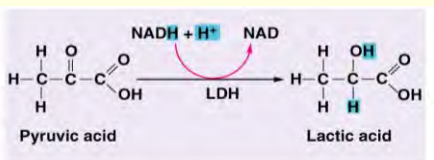
Glycolysis – Step 1

- Is metabolic pathway by which glucose is converted into 2 pyruvates
- Does not require oxygen
- Overall net equation is:
  - glucose + 2NAD + 2ADP + 2P_i → 2 pyruvates + 2NADH + 2 ATP
- Glycolysis is exergonic - produces net of 2ATPs and 2NADHs
- However, glucose must be activated with 2ATPs (phosphorylation) before energy can be obtained
  - Phosphorylation traps glucose inside cell

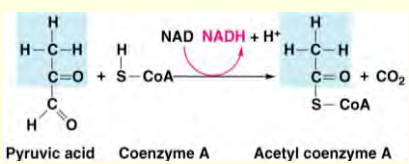


Glycolysis – Step 1

- Pyruvate then undergoes either:
  - 1. anaerobic or
  - 2. aerobic respiration
- To avoid end-product inhibition NADHs produced in glycolysis need to give H^+ away
  - In absence of O_2 NADH gives its H^+ to pyruvate creating lactic acid (anaerobic respiration or fermentation)
    - Makes muscles feel fatigued
    - Lactate production
    - 2 ATPs produced



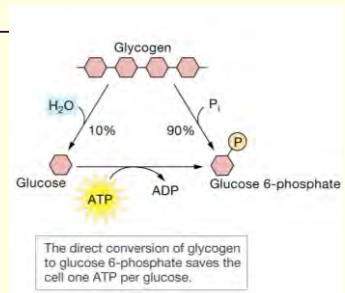
Anaerobic Respiration

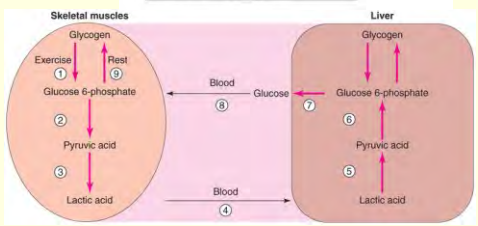


Aerobic Respiration

Glycogenesis and Glycogenolysis

- For osmotic reasons cells can't store many free glucose molecules
  - Instead store glucose as glycogen (glycogenesis)
  - Skeletal muscle and liver store lots of glycogen
  - Glycogenolysis clips glucose out of glycogen as glucose 6-phosphate
    - Phosphate groups trap molecules in cells
- Some skeletal muscle lactic acid goes to liver (Cori Cycle – Removal of Lactic Acid)
  - Where it is converted back through pyruvate to glucose and glycogen
    - Called gluconeogenesis
      - Also can happen with amino acids and glycerol

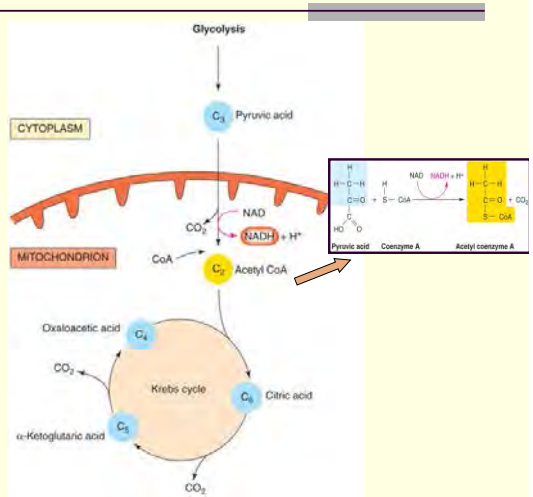




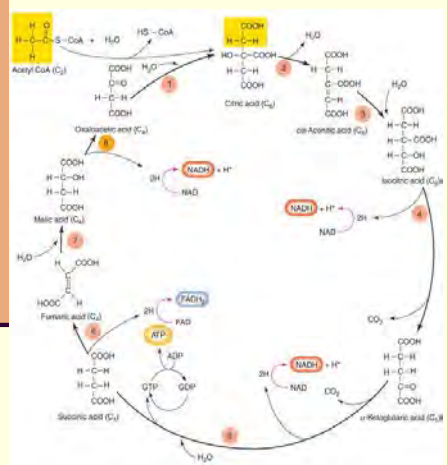
Adopted from Silverthorn 2010

Aerobic Conditions – Krebs Cycle -Step 2

- Begins with acetyl CoA combining with oxaloacetic acid to form citric acid
- In a series of reactions citric acid converted back to oxaloacetic acid to complete the pathway
- Produces:
  - **1 ATP, 3 NADH, and 1 $FADH_2$**
  - NADH and $FADH_2$ carry electrons to Electron Transport Chain (ETC)



Krebs Cycle (Citric Acid Cycle)

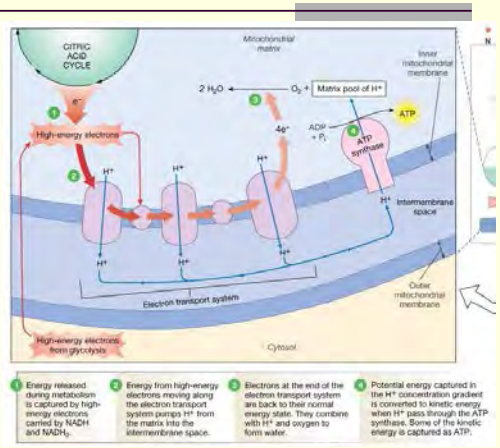


Adopted from Silverthorn 2010

- One turn of the Citric Acid Cycle
  - Each pyruvate => 1ATP,3 NADH & 1FADH
- **One glucose** in aerobic respiration so far:
  - **4 ATP** (2 glycolysis, 2 Krebs Cycle)
  - **10 NADH + H^+** (2 glycolysis, 2 pyruvate/acetyl CoA, 6 Krebs cycle)
  - **2 $FADH_2$** (2 Krebs cycle)

Electron Transport - Step 3

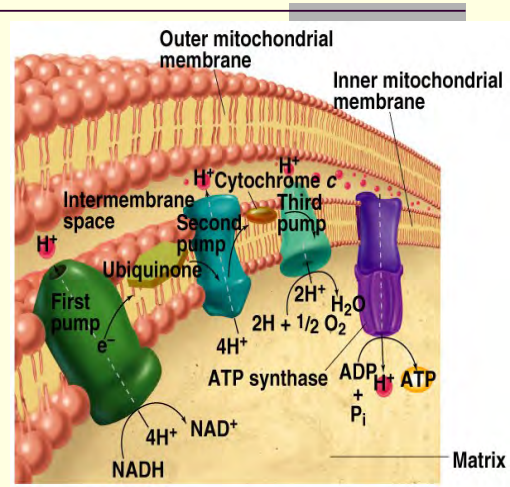
- NADH and $FADH_2$ from Krebs carry electrons to ETC
  - Which are then shuttled in sequence through ETC
  - NAD and FAD are regenerated to shuttle more electrons from Krebs Cycle to ETC
- As each protein in ETC accepts electrons it is reduced
  - When it gives electrons to next protein it is oxidized
  - This process is exergonic
    - Energy is used to phosphorylate ADP to make ATP
    - Called **oxidative phosphorylation**



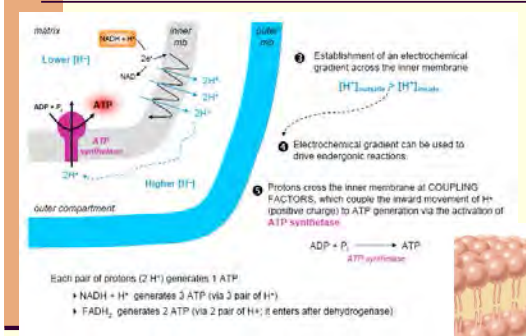
Adopted from Silverthorn 2010

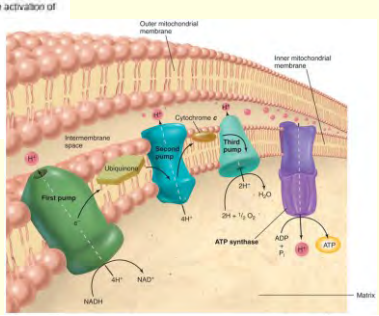
Coupling Electron Transport to ATP Production

- Oxygen functions as the last electron acceptor.
  - Oxidizes cytochrome a_3.
- Oxygen accepts 2 e^-.
- $O_2 + 4\ e^- + 4\ H^+ \rightarrow 2\ H_2O$

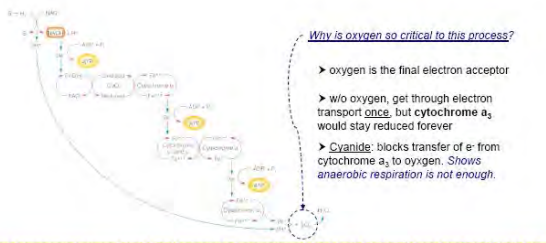


Chemiosmotic Coupling





Summary of Glucose Oxidation



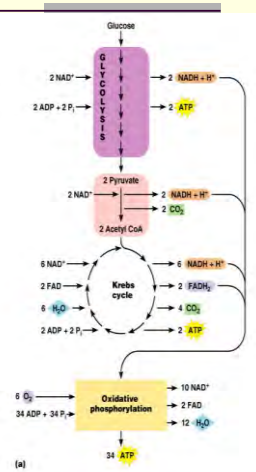
Why is oxygen so critical to this process?

- oxygen is the final electron acceptor
- w/o oxygen, get through electron transport once, but **cytochrome a_3** would stay reduced forever
- Cyanide: blocks transfer of e^- from cytochrome a_3 to oxygen. *Shows anaerobic respiration is not enough.*

phase of respiration	ATP produced directly	reduced coenzymes	ATP via oxidative phosphorylation*
glycolysis	2 ATP	2 NADH+H+	6 ATP (or 4*)
pyruvate to acetyl-CoA		2 NADH+H+	6 ATP
citric acid cycle (1 glucose → 2 turns)	2 ATP	6 NADH+H+ 2 $FADH_2$	18 ATP 4 ATP
	4 ATP		34 ATP (or 32*)

Total ATP/glucose = 38 ATP

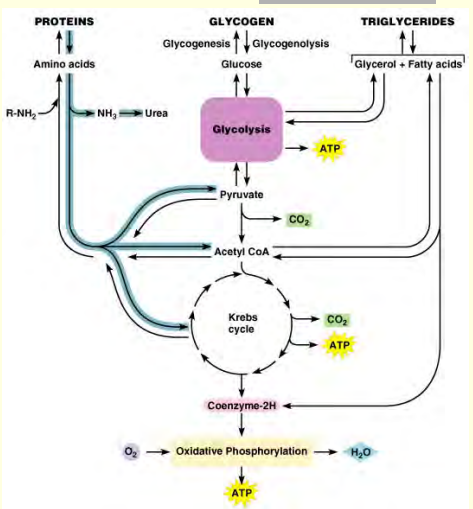
*produced in cytoplasm, get 4 or 6 ATP depending upon route of entry of NADH into mitochondria



BKG: cellular metabolism 77

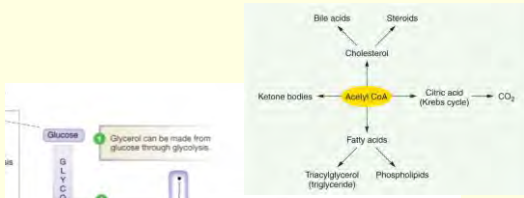
Lipid & Protein Metabolic Pathways
How it is all connected

- Fats can be hydrolyzed to glycerol and fatty acids
  - These can be modified to run through Krebs
- Proteins can be broken down to amino acids
  - Which can be deaminated and run through Kreb's
- These pathways can be used to interconvert carbohydrates, fats, and proteins
- When more energy is taken in than consumed, ATP synthesis is inhibited
- Glucose converted into glycogen and fat



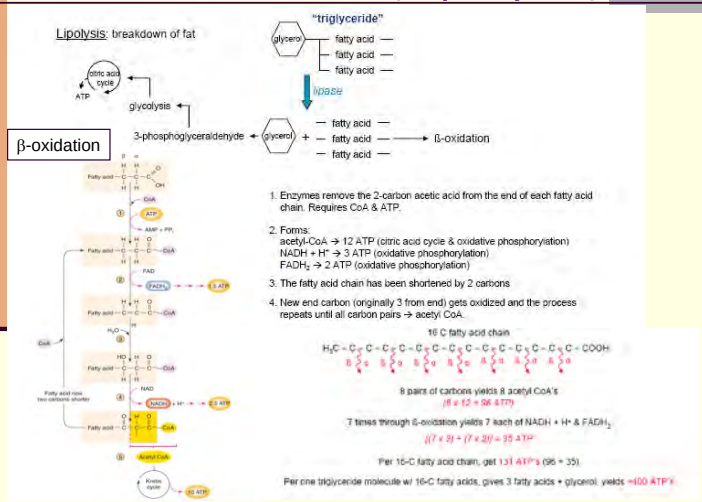
2. lipid pathways
Fat Synthesis (Lipogenesis)

- Acetyl CoAs can be used to form fatty acids
  - Fatty acids + glycerol = Fat (triglycerides)
    - Occurs mainly in adipose and liver tissues
- Fat is major form of energy storage in body
  - Yields 9 kCal/g
  - Carbos and proteins yield only 4kCal/g



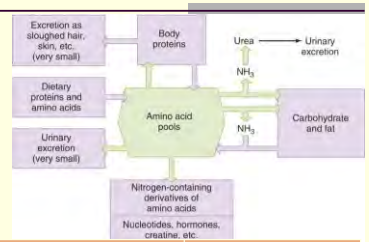
Adopted from Silverthorn 2010

2. lipid pathways
Fat Breakdown (Lipolysis)



3. protein pathways
Amino Acid Metabolism

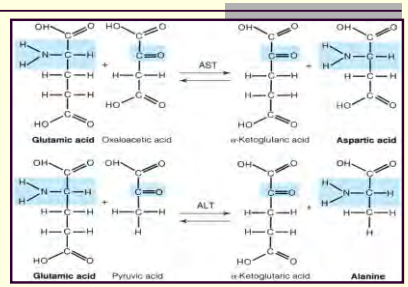
- Nitrogen (N) is ingested primarily as protein
  - Which is used in body as amino acids
- Excess is excreted mainly as urea
- Nitrogen balance = N ingested minus N excreted
  - Positive N balance: more N ingested than excreted
  - Negative N balance: less N ingested than excreted
- In healthy adults amount of N excreted = amount ingested
- Excess amino acids can be converted into carbs and fat



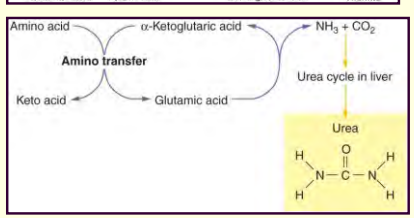
Essential Amino Acids	Nonessential Amino Acids
Arginine	Aspartatic Acid
Histidine	Glutamatic Acid
Isoleucine	Proline
Leucine	Glycine
Lysine	Serine
Methionine	Alanine
Phenylalanine	Arginine
Tryptophane	Asparagine
Threonine	Glutamine
Valine	Tyrosine

3. protein pathways

- **Transamination**
- New amino acids can be obtained by transamination
  - Which is addition of -NH_2 to pyruvate or Krebs cycle ketones to make a new amino acid
  - Requires transaminase and vitamin B6



- **Oxidative Deamination**
- Is process by which excess amino acids are eliminated
- -NH_2 is removed from glutamic acid, forming keto acid and ammonia
  - Ammonia is converted to urea and excreted
  - Keto acid goes to Krebs or to fat or glucose



How it is all connected

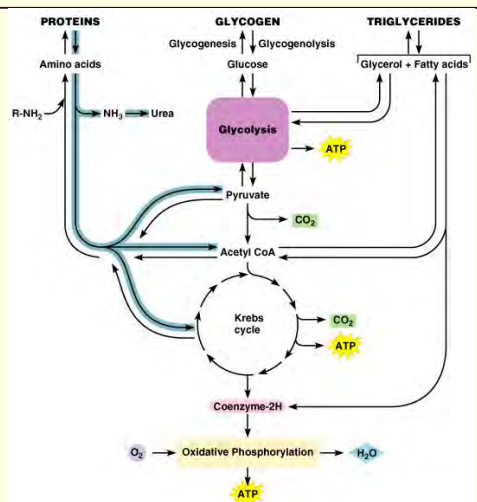


Table 4.4 Examples of Inborn Errors in the Metabolism of Amino Acids, Carbohydrates, and Lipids

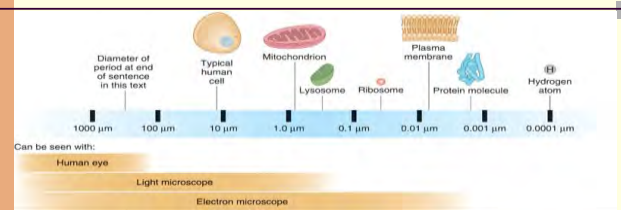
Metabolic Defect	Disease	Abnormality	Clinical Result
Amino acid metabolism	Phenylketonuria (PKU)	Increase in phenylpyruvic acid	Mental retardation, epilepsy
	Albinism	Lack of melanin	Susceptibility to skin cancer
	Maple-syrup disease	Increase in leucine, isoleucine, and valine	Degeneration of brain, early death
	Homocystinuria	Accumulation of homocystine	Mental retardation, eye problems
Carbohydrate metabolism	Lactose intolerance	Lactose not utilized	Diarrhea
	Glucose 6-phosphatase deficiency (Gierke's disease)	Accumulation of glycogen in liver	Liver enlargement, hypoglycemia
	Glycogen phosphorylase deficiency	Accumulation of glycogen in muscle	Muscle fatigue and pain
Lipid metabolism	Gaucher's disease	Lipid accumulation (glucocerebroside)	Liver and spleen enlargement, brain degeneration
	Tay-Sachs disease	Lipid accumulation (ganglioside G_{M2})	Brain degeneration, death by age 5
	Hypercholestremia	High blood cholesterol	Atherosclerosis of coronary and large arteries

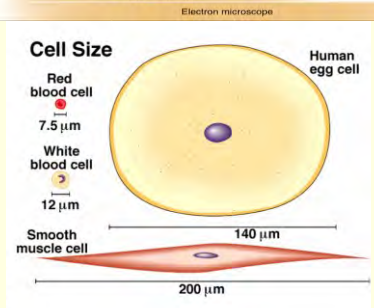
Cell structure and function

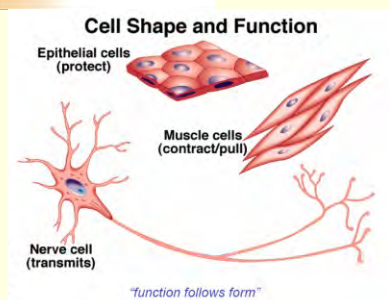
Cell Structure and Protein Function

- **I. Cell Membrane**
  - Structure ("Fluid Mosaic Model")
  - Transport properties of membranes
    - **Diffusion**
      - Simple
      - Facilitated
      - Osmosis
    - **ActiveTransport**
      - Primary
      - Cotransport
    - **Exocytosis/Endocytosis**
      - Phagocytosis
      - Pinocytosis
      - Receptor-mediated endocytosis
      - Potocytosis
      - Transcytosis
  - **II. Cytoplasm**
  - Cytosol
  - Organelles
    - Nonmembraneous
    - Membraneous
  - **III. Nucleus**

The scale of things





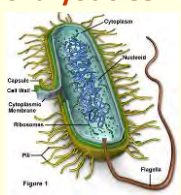


Structure of a Generalized Cell

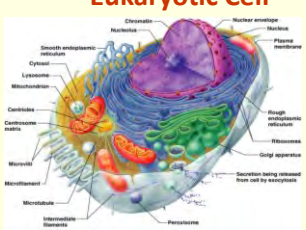
- ***Prokaryotic cells***
  - Thought to be the first cells to evolve
  - Lack a nucleus
  - Represented by bacteria and archaea
- ***Eukaryotic cells***
  - Have a nucleus that houses DNA
  - Many membrane-bound organelles

- **What have prokaryotes and eukaryotes in common**
  - A plasma membrane that surrounds and delineates the cell
  - A cytoplasm that is the semi-fluid portion inside the cell that contains organelles
  - DNA

Prokaryotic Cell

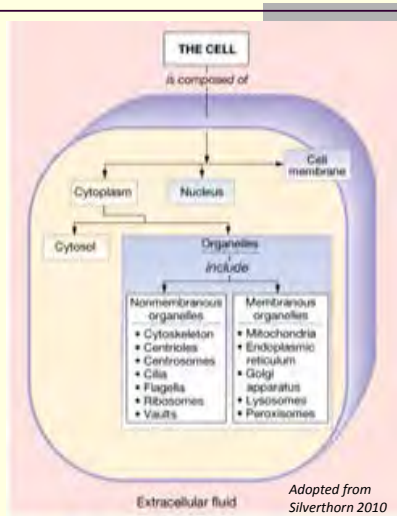


Eukaryotic Cell



Structure of a Generalized Cell

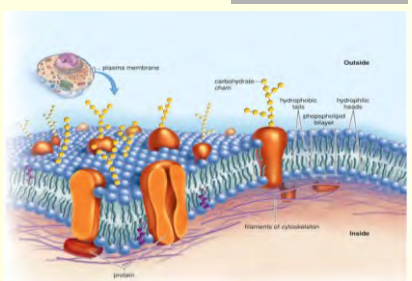
- Cell membrane
- Nucleus
- Cytoplasm
  - Cytosol
  - Organelles
  - Inclusion
    - Dissolved
    - Insoluble



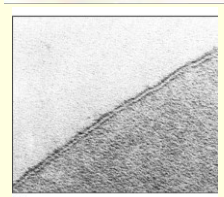
Adopted from Silverthorn 2010

I. Cell Membrane - Structure

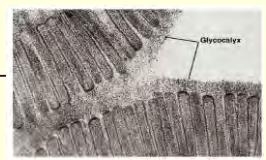
- Surrounds and gives cell form; is selectively permeable
- **Fluid-mosaic model:**
- It is a **phospholipid bilayer**
- It is embedded with proteins that move in space
- It contains **cholesterol** for support
  - Helps to decrease permeability to H_2O
  - Insures membrane flexibility through variety of temperatures and conditions
- It contains carbohydrates on proteins and lipids
- Selectively permeable



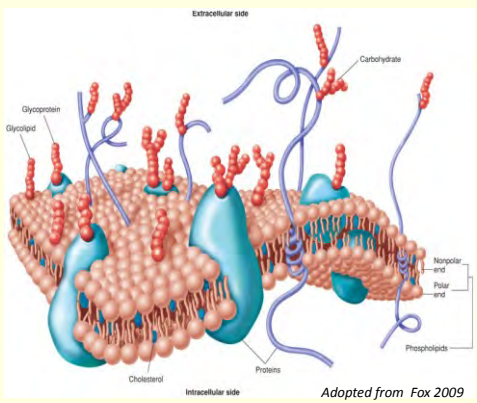
Adopted from Fox 2009



Plasma Membrane



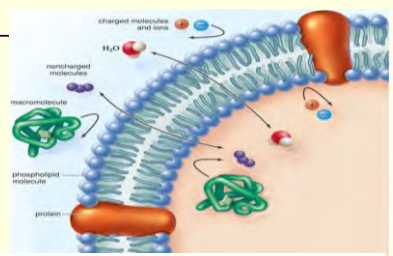
- ***Carbohydrates*** in form of glycoproteins and glycolipids are part of outer surface
  - Impart negative charge to surface
  - Functions:
    - Cell to cell recognition
    - Can serve as cell surface markers (antigens)
    - Receptors
- ***Proteins*** customize membranes
  - Provide structural support
  - Serve as transporters, enzymes, receptors and identity markers

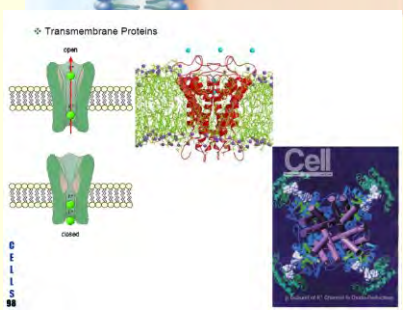


Adopted from Fox 2009

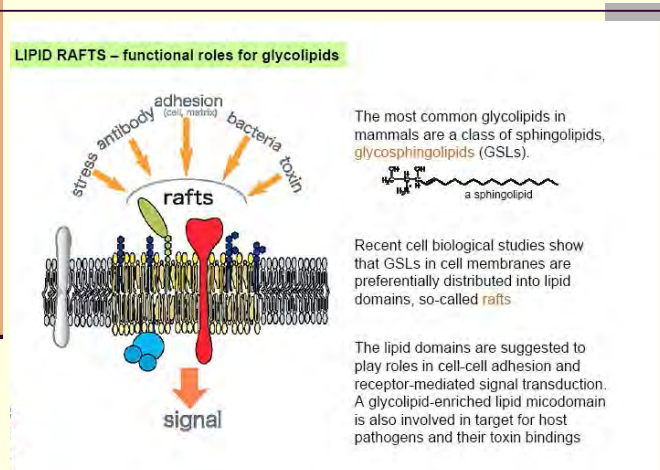
What does selectively permeable mean?

- The membrane allows some things in while keeping other substances out
- What can get through:
  - Lipid soluble compounds – alcohol, steroids
  - CO_2, O_2
  - Nonpolar molecules
  - H_2O
- How do things move across the plasma membrane?
  - Diffusion
  - Osmosis
  - Facilitated transport
  - Active transport
  - Bulk Transport
    - Endocytosis and exocytosis



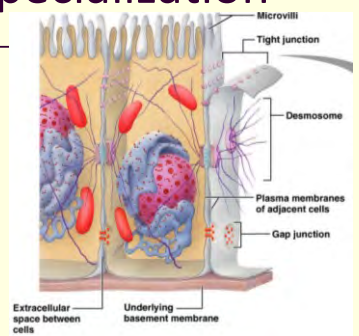


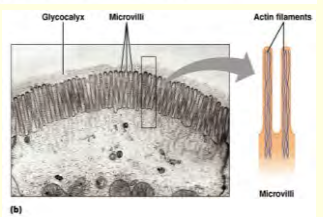
Cell Membrane - Structure



Cell Membrane - Specialization

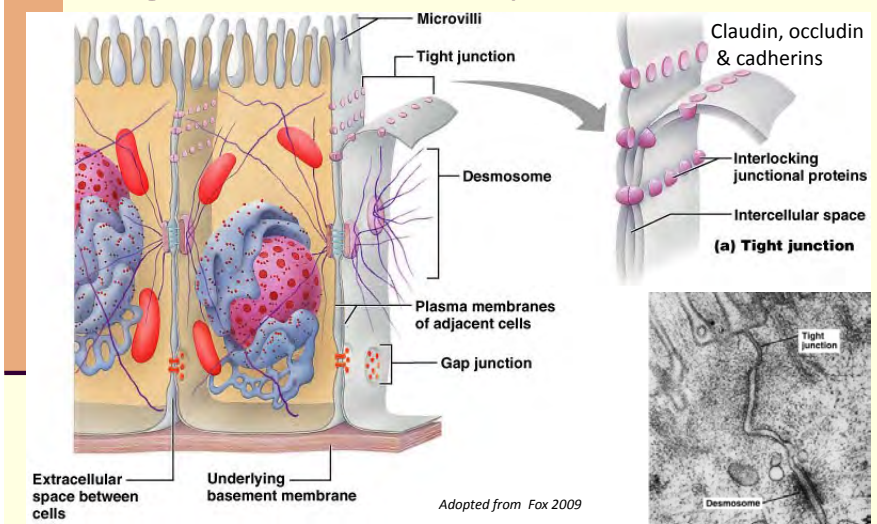
- Increase the plasma membrane surface area – in **absorptive cells - epithelia**
  - Microvilli
- **Cell to cell adhesions**
  - Gap junctions
  - Tight junctions
  - Desmosomes





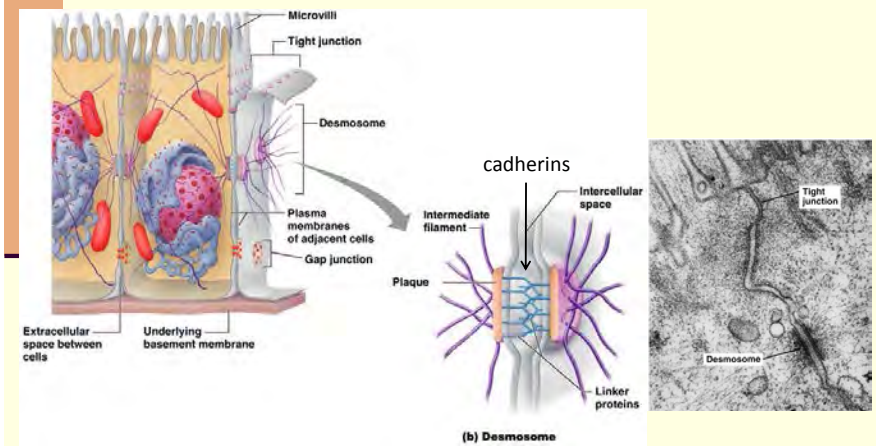
Adopted from Fox 2009

Tight Junctions – epithelial cells

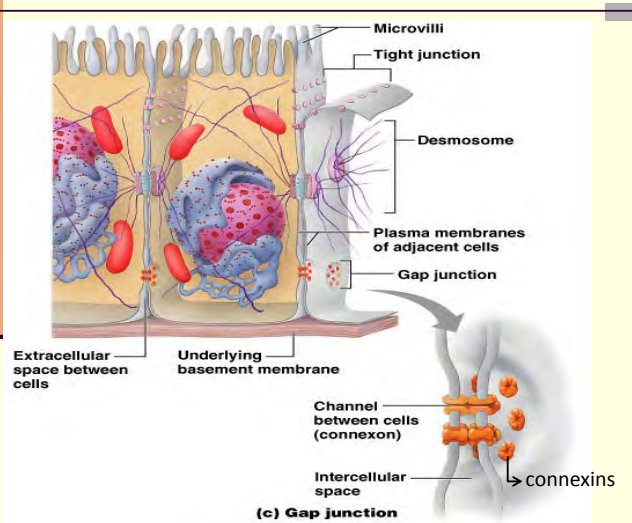


Adopted from Fox 2009

Desmosomes – in tissues subjected to mechanical stress



Gap Junctions – electrically excitable tissues



Cell Membrane - Transport properties of membranes – next lecture

- Diffusion
  - Simple
  - Facilitated
  - Osmosis
- ActiveTransport
  - Primary
  - Cotransport
- Exocytosis/Endocytosis
  - Phagocytosis
  - Pinocytosis
  - Receptor-mediated endocytosis
  - Potocytosis
  - Transcytosis

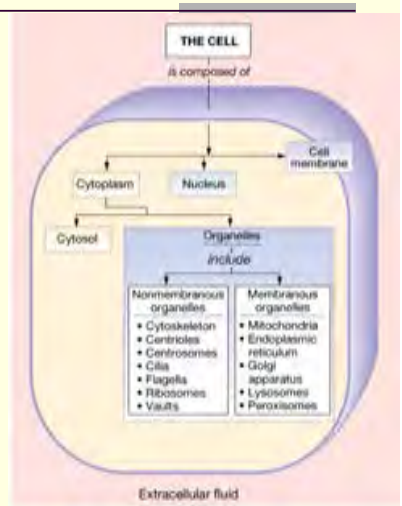
II. Cytoplasm
Region of the cell outside of nucleus

Cytosol – fluid portion of the cytoplasm outside of the organelles

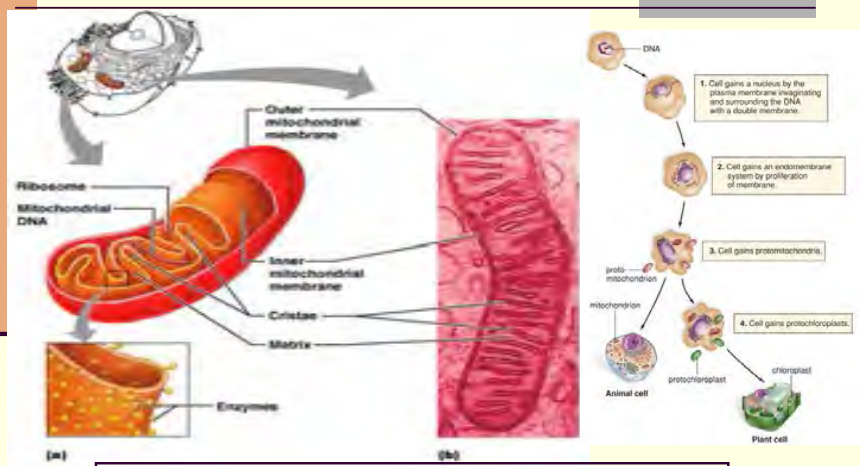
Organelles

- Membraneous
- Nonmembraneous

Inclusions – glycogen granules, lipid droplets, pigments

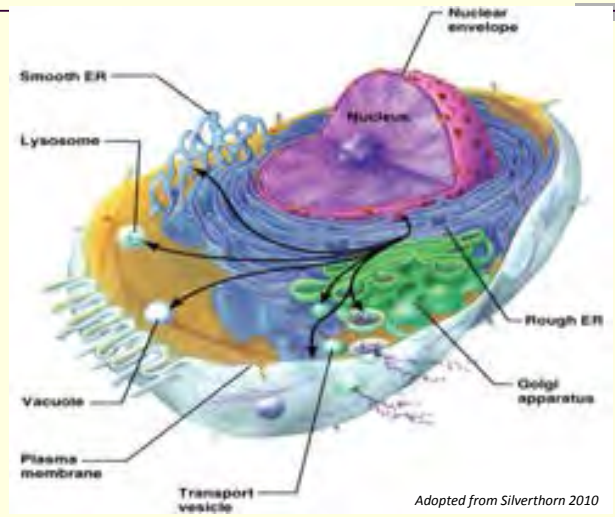


Mitochondria –power plants of the cell

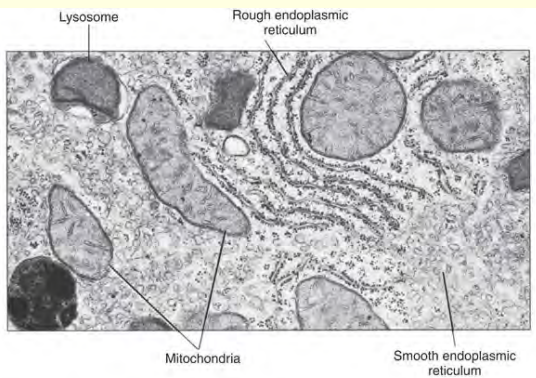


Complex organelles – contain their own DNA and RNA and are able to reproduce themselves

Endomembrane System



Adopted from Silverthorn 2010



Rough endoplasmic reticulum

Structure: Extensive membranous network of flattened sacs. Encloses a space that is continuous throughout the organelle and with the space between the two nuclear-envelope membranes. Has ribosomal particles attached to its cytosolic surface.

Function: Proteins synthesized on the attached ribosomes enter the lumen of the reticulum from which they are ultimately distributed to other organelles or secreted from the cell.

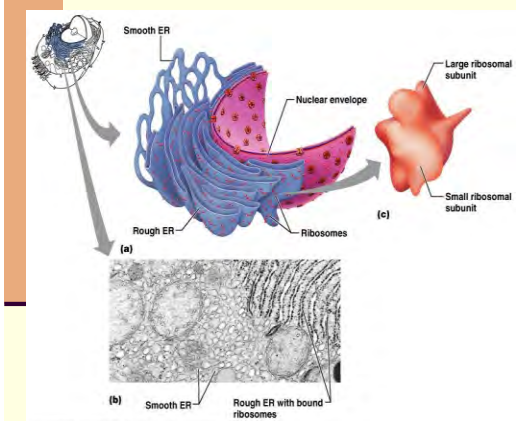
Smooth endoplasmic reticulum

Structure: Highly branched tubular network that does not have attached ribosomes but may be continuous with the rough endoplasmic reticulum.

Function: Contains enzymes for fatty acid and steroid synthesis. Stores and releases calcium, which controls various cell activities.

Adopted from Silverthorn 2010

Endoplasmic Reticulum (ER) – Continuation of the nuclear membrane

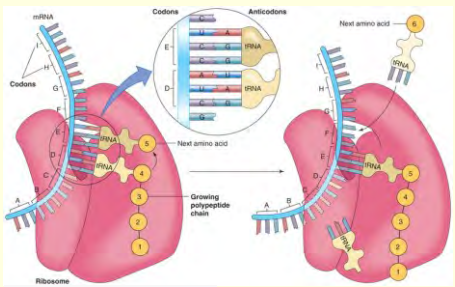


Adopted from Silverthorn 2010

- **Rough ER – protein synthesis**
  - phospolipids synthesis
- **Smooth ER – lipid metabolism**
  - cholesterol synthesis
  - steroid hormone synthesis
  - detoxification (liver, kidney)
    - *drug tolerance*
  - breakdown of glycogen

Protein Synthesis

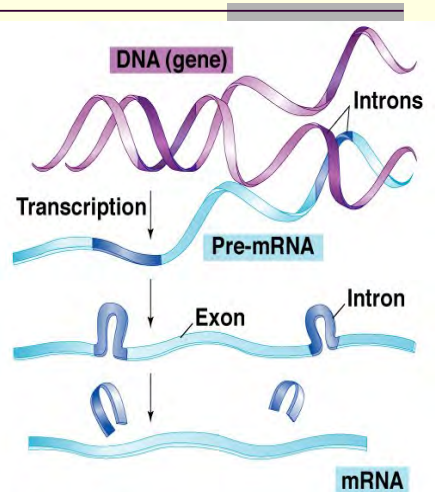
- Occurs one amino acid at a time according to sequence of base triplets in mRNA
- In cytoplasm, mRNA attaches to ribosomes forming a **polysome** where translation occurs
- In a ribosome, anticodons on tRNA bind to mRNA codons
- Amino acids on adjacent tRNAs are brought together and linked enzymatically by peptide bonds
  - This forms a polypeptide; at a stop codon it detaches from ribosome



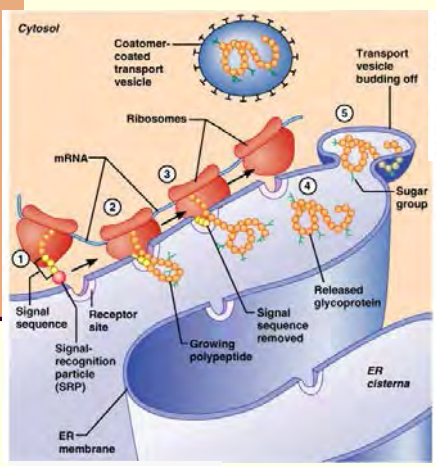
Adopted from Silverthorn 2010

Types of RNA

- 4 types of RNA produced within nucleus by transcription.
  - **Precursor mRNA** pre-mRNA):
    - Altered in nucleus to form mRNA.
  - **Messenger RNA (mRNA):**
    - Contains the code for synthesis of specific proteins.
  - **Transfer RNA (tRNA):**
    - Decodes genetic message contained in mRNA.
  - **Ribosomal RNA (rRNA):**
    - Forms part of the ribosome structure.



Signal Mechanism of Protein Synthesis

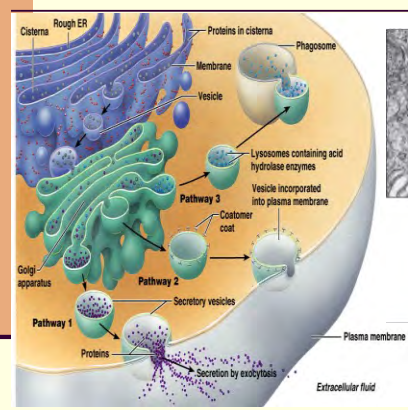


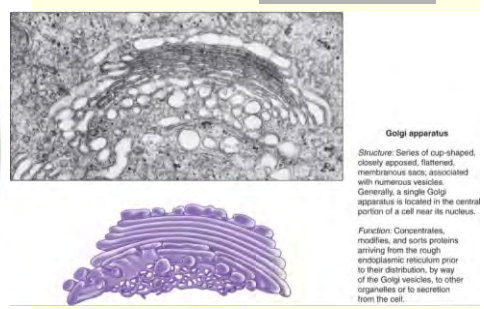
Adopted from Fox 2009

- Proteins to be secreted are made in ribosomes of rough ER
  - Contain a leader sequence of 30+ hydrophobic amino acids that directs such proteins to enter cisternae of ER
    - Where leader sequence is removed; protein is modified

Role of the Golgi Apparatus

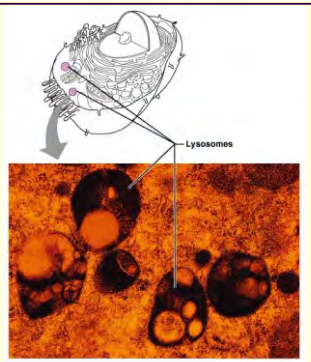
Trafficking of cellular proteins – modification, sorting, concentrating, and packaging





Adopted from Silverthorn 2010

Lysosomes - cellular stomachs



Adopted from Silverthorn 2010

- Are vesicle-like organelles containing digestive enzymes and matter being digested
  - Involved in recycling cell components
  - Involved in programmed cell death
- **Functions:**
- digesting particles
- degrading organells
- metabolism – breaking down glycogen
- breaking down tissues
- breaking down bone

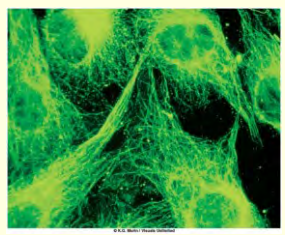
- ***Genetic illnesses***
- **Tay-Sachs disease** is a fatal genetic lipid storage disorder caused by insufficient activity of an enzyme called *beta-hexosaminidase A* that catalyzes the biodegradation of acidic fatty materials
  - http://www.ninds.nih.gov/disorders/taysachs/taysachs.htm
- **Gaucher's disease** - an inherited metabolic disorder in which harmful quantities of a fatty substance called *glucocerebroside* accumulate in the spleen, liver, lungs, bone marrow, and sometimes in the brain . It is caused by a deficiency of an enzyme called *glucocerebrosidase*.
  - http://www.ninds.nih.gov/disorders/gauchers/gaucher s.htm

Peroxisomes

- Are vesicle-like organelles containing oxidative enzymes
  - Involved in detoxification in liver -removal of hydrogen and H_2O_2

Organelles

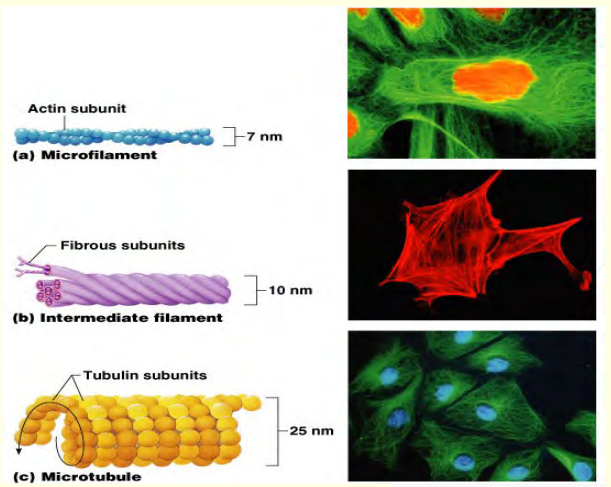
- Nonmembraneous
  - **ribosomes** – protein factories
  - **endosomes** – further trafficking of cellular proteins
  - **cytoskeleton** – maintain cell shape and cell movement



- **Cytoskeleton** is a latticework of microfilaments and microtubules filling cytoplasm
  - Gives cell its shape and structure
  - Forms tracks upon which things are transported around cell

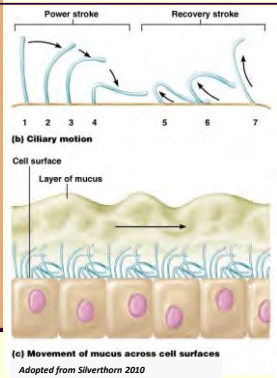
Adopted from Silverthorn 2010

Cytoskeleton – maintains cell shape and cell movement

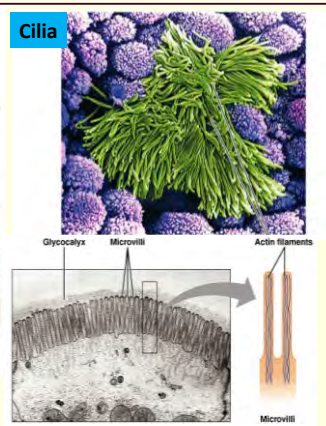


Adopted from Silverthorn 2010

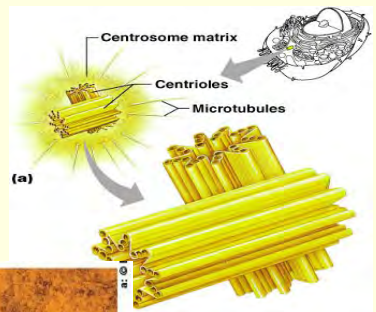
Cilia & Flagella



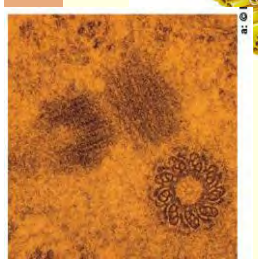
Adopted from Silverthorn 2010



Centrosome and Centrioles



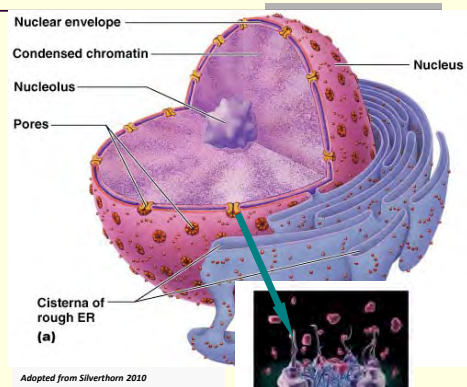
Adopted from Silverthorn 2010



- All animal cells have a **centrosome** located near nucleus in interphase
  - Microtubule organizing center
  - Contains 2 centrioles
- Basal bodies generating microtubules and mitotic spindle

III. Cell nucleus and genetic control

- **Nucleus**
  - most cells have single nucleus
  - contains genetic material
  - **controls all cell processes:**
    - Gene expression
    - Protein synthesis
    - Cell division
    - Cell death

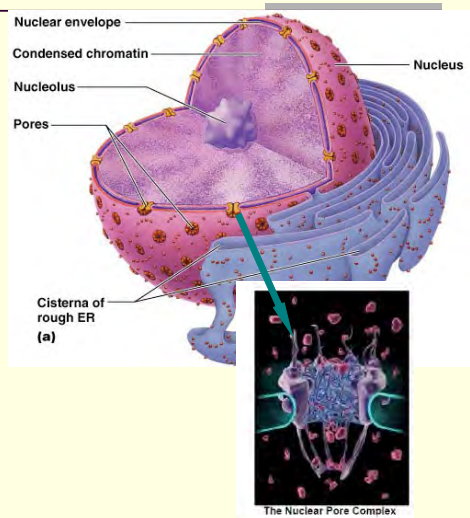


Adopted from Silverthorn 2010


The Nuclear Pore Complex

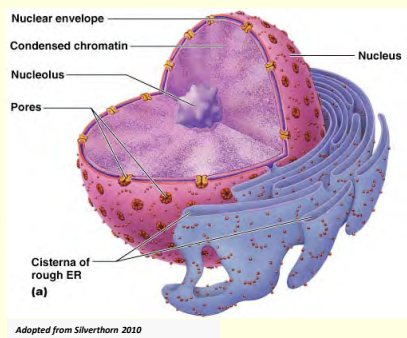
Cell nucleus and genetic control

- **Nuclear envelope** – separates nucleus from cytoplasm
  - Two membrane structure - inner and outer membrane
    - Outer membrane is continuous with ER.
- **Nuclear pore** - complexes fuse inner and outer membranes together.
  - Communication between cytosol and nucleus
  - Permeable for ions and small molecules
  - Selective active transport of proteins and RNA – requires energy
    - Regulation of gene expression.
      - Transport of mRNA out of nucleus to ribosomes.

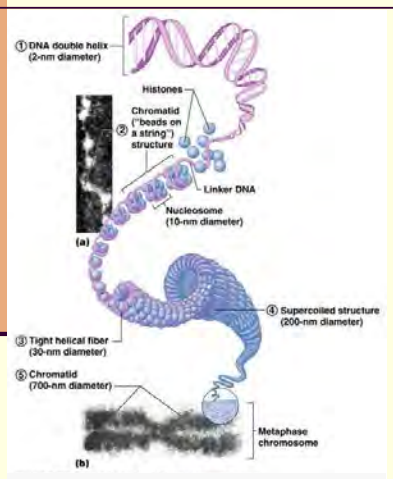


Cell nucleus and genetic control

- **Chromatin**
  - Forms in non dividing cells
  - loosely organized DNA with proteins (histons)
    - Thread-like material that makes up the chromosomes during cell division
- **Nucleolus:**
  - Contains complex of DNA,RNA and proteins
  - DNA contains the genes that code for the production of mRNA.



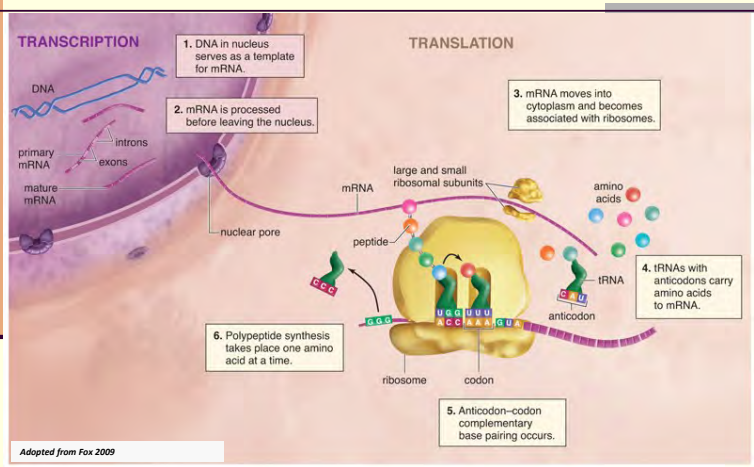
Chromatin and chromosome structure



Adopted from Silverthorn 2010

- Is made of DNA and its associated proteins (=**histones**)
- Histones are positively charged and form spools around which negatively charged DNA strands wrap
  - Each spool and its DNA is called a **nucleosome**
- Euchromatin is the part of chromosomes active in transcription
  - Light in color
- Heterochromatin is highly condensed region where genes are permanently inactivated
  - Darker in color

Cell nucleus controls of all cell processes



Adopted from Fox 2009

Cellular Components: Structure & Function

Component	Structure	Function
Plasma (cell) membrane	Membrane composed of double layer of phospholipids in which proteins are embedded	Gives form to cell and controls passage of materials into and out of cell
Cytoplasm	Fluid, jellylike substance between the cell membrane and the nucleus in which organelles are suspended	Serves as matrix substance in which chemical reactions occur
Endoplasmic reticulum	System of interconnected membrane-forming canals and tubules	Agranular (smooth) endoplasmic reticulum metabolizes nonpolar compounds and stores Ca^{2+} in striated muscle cells, granular (rough) endoplasmic reticulum assists in protein synthesis
Ribosomes	Granular particles composed of protein and RNA	Synthesize proteins
Golgi complex	Cluster of flattened membranous sacs	Synthesizes carbohydrates and packages molecules for secretion, secretes lipids and glycoproteins
Mitochondria	Membranous sacs with folded inner partitions	Release energy from food molecules and transform energy into usable ATP
Lysosomes	Membranous sacs	Digest foreign molecules and worn and damaged organelles
Peroxisomes	Spherical membranous vesicles	Contain enzymes that detoxify harmful molecules and break down hydrogen peroxide
Centrosome	Nonmembranous mass of two rodlike centrioles	Helps to organize spindle fibers and distribute chromosomes during mitosis
Vacuoles	Membranous sacs	Store and release various substances within the cytoplasm
Microfilaments and microtubules	Thin, hollow tubes	Support cytoplasm and transport materials within the cytoplasm
Cilia and flagella	Minute cytoplasmic projections that extend from the cell surface	Move particles along cell surface or move the cell
Nuclear envelope	Double-layered membrane that surrounds the nucleus, composed of protein and lipid molecules	Supports nucleus and controls passage of materials between nucleus and cytoplasm
Nucleolus	Dense nonmembranous mass composed of protein and RNA molecules	Produces ribosomal RNA for ribosomes
Chromatin	Fibrous strands composed of protein and DNA	Contains genetic code that determines which proteins (including enzymes) will be manufactured by the cell

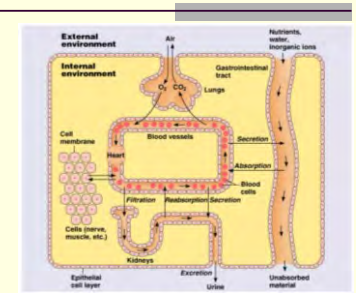
Interactions Between Cells and the Extracellular Environment

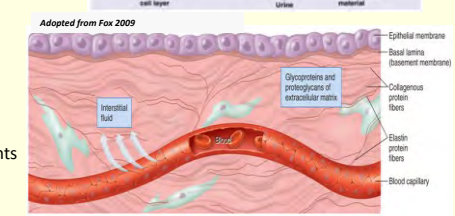
Interactions Between Cells and the Extracellular Environment

- Extracellular enviroment
- Categories of transport across the plasma
- Membrane diffusion and osmosis
- Membrane potential
- Cell signaling

Extracellular Environment

- Includes all constituents of body outside cells
- **Body Fluid Compartments** - 67% of total body H_2O is inside cells (=intracellular compartment); 33% is outside cells (=extracellular compartment-ECF)
  - 20% of ECF is blood plasma
  - 80% of ECF is interstitial fluid contained in gel-like matrix
- **Extracellular Matrix** - Is a meshwork of collagen and elastin fibers linked to molecules of gel-like ground substance and to plasma membrane integrins = glycoprotein adhesion molecules that link intracellular and extracellular compartments

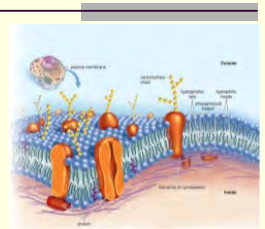




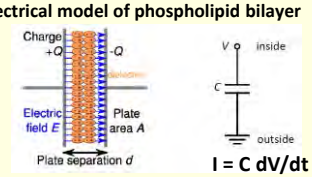
Plasma Membrane Characteristics

- Plasma membrane is selectively permeable - allows only certain kinds of molecules to pass due to it´s characteristics
- Composed of phospholipid bilayer and proteins, and some carbohydrates
- Overall it has hydrophobic properties
- **Conductance *g = 1/R***
- **Capacitance *C = Q/V*** - because the membrane is so thin ($\approx 6\times10^{-9}m$), we don't need much voltage to separate the charges and therefore the membrane capacitance is quite high; per unit area, it is

$$c = C/S \approx 10^{-2} Fm^{-2}$$



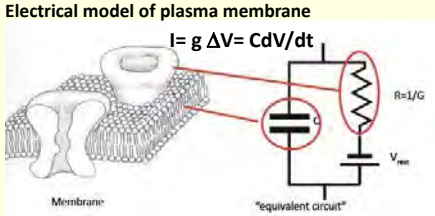
Electrical model of phospholipid bilayer



I = C dV/dt

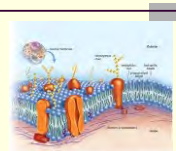
Electrical model of plasma membrane

I= g ΔV= CdV/dt



Transport Across Plasma Membrane

- Transport Categories Across the Plasma Membrane:
  - **Passive transport** moves compounds down concentration gradient; requires no energy
  - **Active transport** moves compounds against a concentration gradient; requires energy and transporters
  - Many important molecules have transporters and channels
    - **Carrier-mediated transport** involves specific protein transporters
    - **Non-carrier mediated transport** occurs by diffusion



Composition of Extracellular and Intracellular Fluids

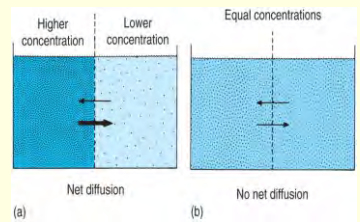
	Extracellular [mM]	Intracellular [mM]
Na^+	150	15
K^+	5	150
Ca^{2+}	1	0.0001
Mg^{2+}	1.5	12
Cl^-	110	10
HCO_3^-	24	10
P_i	2	40
Amino acids	2	8
Glucose	5.6	1
ATP	0	4
Proteins	0.2	4

Diffusion and Osmosis

- Diffusion Through the Plasma Membrane
  - Nonpolar (Lipid) Molecule Movement
  - Inorganic Ion Movement
- Rate of Diffusion
- Osmosis
- Regulation of Blood Osmolality
- Osmotic Pressure

Diffusion – Passive Transport

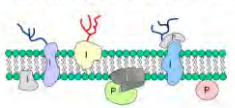
- Is random motion of molecules (gases and liquids)
  - Net movement is from region of high to low concentration
  - It obeys *2nd Law of Thermodynamics*: "***systems will change spontaneously from states of low probability to states of high probability***"
    - $\Delta S = R \ln p_B/p_A$ (*S – entropy*)
    - $-\Delta G = -\Delta H + T\Delta S$
      - H = E + PV (*H = enthalpy*)
      - *G = Gibbs free energy*
- **Factors affecting rate of *net* diffusion:**
  - 1. DC -concentration gradient
  - 2. Size of diffusing substance (smaller = faster)
  - 3. Permeability of membrane to it
  - 4. Temperature
  - 5. Surface area of membrane



Fick's Law of Diffusion:

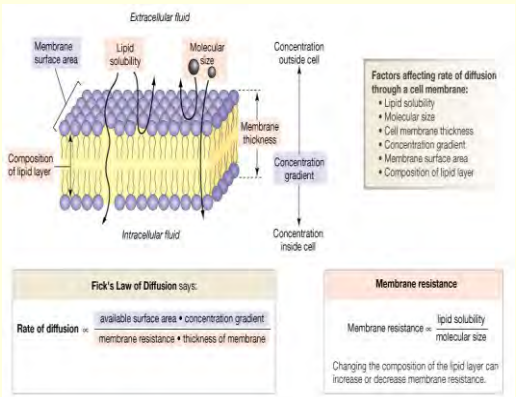
$$\text{diffusion rate} \propto \frac{\text{concentration diff.} \times \text{surface area} \times T^{\circ}}{\sqrt{\text{molecular weight}} \times \text{distance}}$$

BUT! Fick's Law assumes permeability of barrier is not restrictive



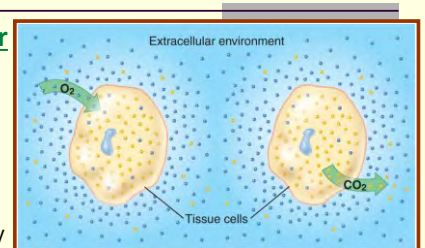
Factors Affecting Membrane Permeability

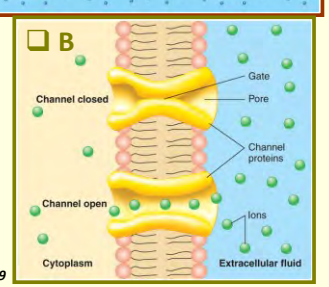
- Lipid-solubility of molecule
  - Polar/non-polar
  - Charged
- Molecular weight and shape of molecule
- Temperature
- Membrane thickness
- Membrane properties
  - Cholesterol
  - Proteins



Diffusion

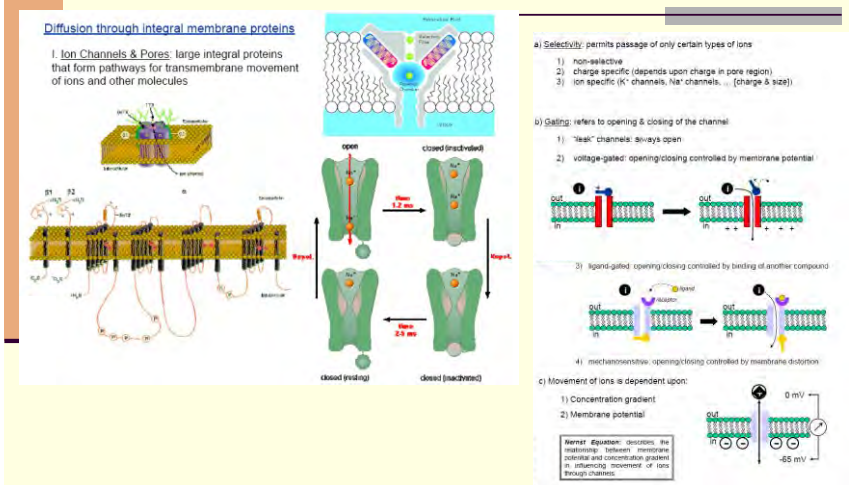
- **Simple Diffusion** - **Non-polar compounds** readily diffuse thru cell membrane
  - O_2, CO_2, fatty acids, water
  - Also some small molecules such as CO_2 and H_2O
    - Gas exchange occurs this way
- **Facilitated Diffusion** - Cell membranes are impermeable to charged and most **polar compounds**
  - Charged molecules must have an **ion channel** or **transporter** to move across membrane





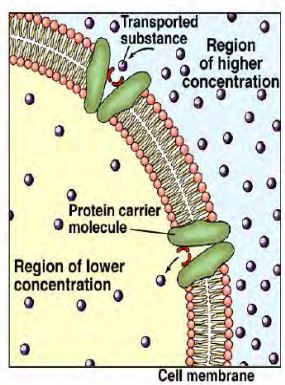
Adopted from Fox 2009

Facilitated Diffusion I.



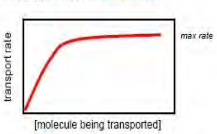
Facilitated Diffusion II.

II. Carriers or Transporters: large integral proteins that function to translocate lipid insoluble molecules ("Facilitated Diffusion")

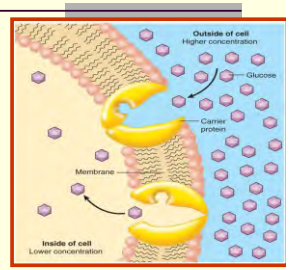


Characteristics:

1. Rate of diffusion depends on:
   a. concentration gradient
   b. concentration of transporter
   c. reaction rate between molecule & carrier
2. Facilitated diffusion is *saturable*



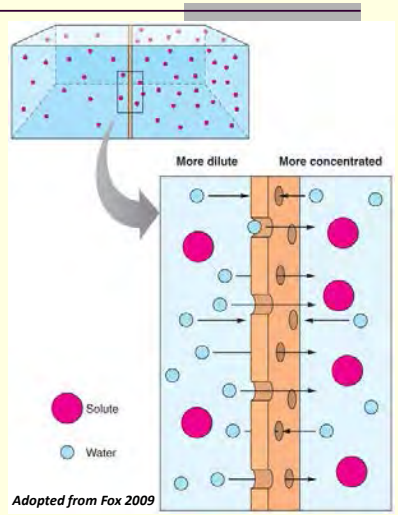
3. Requires no energy



Glucose transport – GLUT transporter

Osmosis – Passive Transport

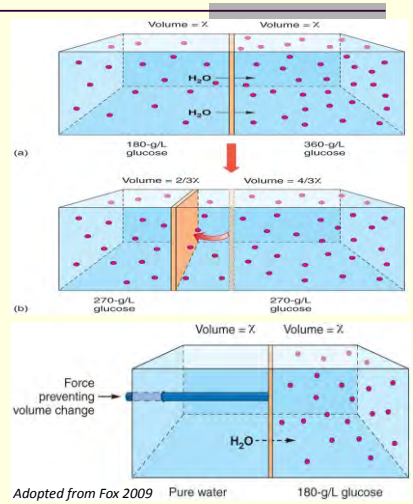
- Is net diffusion of H_2O across a selectively permeable membrane
  - H_2O diffuses down its concentration gradient
  - H_2O is less concentrated where there are more solutes
    - Solutes have to be osmotically active i.e., cannot freely move across membrane



Adopted from Fox 2009

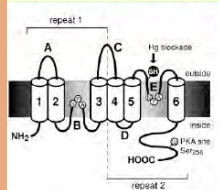
Osmosis – Passive Transport

- H_2O diffuses down its concentration gradient until its concentration is equal on both sides of a membrane
- Some cells have "***water channels***" (**aquaporins**) to facilitate osmosis
- **Osmotic Pressure** - Is the force that would have to be exerted to stop osmosis - Indicates how strongly H_2O wants to diffuse
- **$\pi = CRT$**
  - π – osmotic pressure
  - C – total concentration of solute
  - R – univ. gas constant
  - T – absolute temperature



Adopted from Fox 2009

AQUAPORINS – transport proteins for H_2O

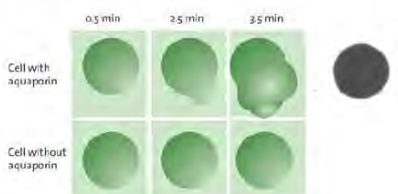


secondary structure of aquaporins

Peter Agre: 2003 Nobel Prize in Chemistry for discovering a class of membrane-spanning proteins that transport water across cell membranes.

AQUAPORINS (water channels) are crucial for a variety of physiological processes in humans including vision and water reabsorption in the kidneys.

In plants, they control water uptake in the roots as well as water distribution to the stalk, leaves, and flowers

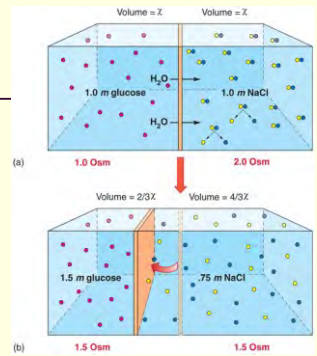


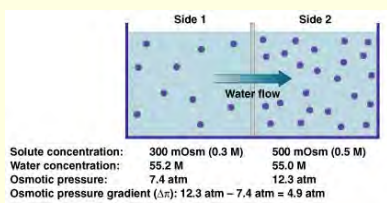
EXPLOSIVE! When placed in distilled water, frog eggs that contain aquaporin take in so much water that they explode. Frog eggs without the protein don't.

Molarity and Molality

- **1 molar solution** (1 M) = 1 mole of solute dissolved in 1L of solution
  - 1 M contain 6.23×10^{23} molecules of compound
  - Doesn't specify exact amount of H_2O
- **1 molal solution** (1.0m) = 1 mole of solute dissolved in 1 kg H_2O

- Osmolality (Osm) is total molality of a solution
  - E.g., **1 mole** of NaCl yields a 2 Osm solution
    - Because NaCl dissociates into Na^+ and Cl^-



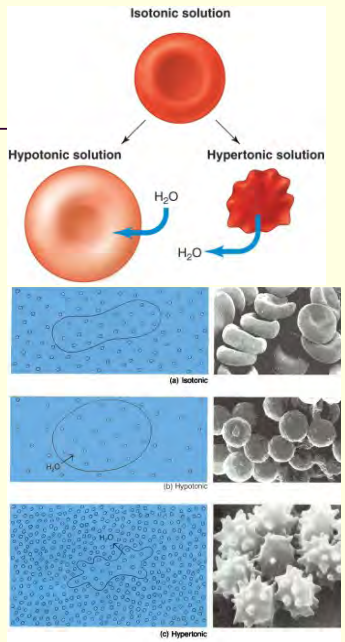


Adopted from Fox 2009

Tonicity

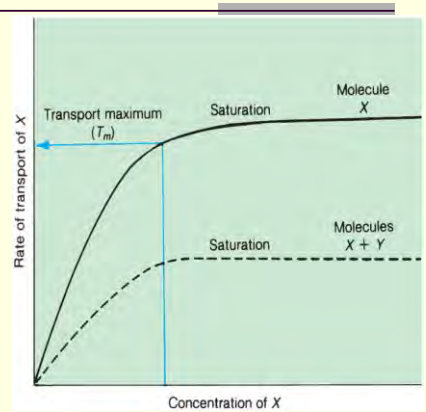
- Is the effect of a solution on osmotic movement of H_2O
- Isotonic solutions have same osmotic pressure
- Hypertonic solutions have higher osmotic pressure and are osmotically active
  - Hypotonics have lower osmotic pressure
- Isosmotic solutions have same osmolality as plasma
- Hypo-osmotic solutions have lower osmotic pressure than plasma
  - Hyper-osmotics have higher pressure than plasma

Adopted from Fox 2009



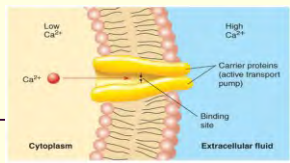
Carrier-Mediated Transport

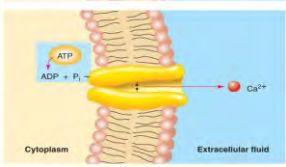
- Molecules too large and polar to diffuse are transported across membrane by *protein carriers*
- Protein carriers exhibit:
  - Specificity for single molecule
  - Competition among substrates for transport
  - Saturation when all carriers are occupied
    - This is called T_m (transport maximum)

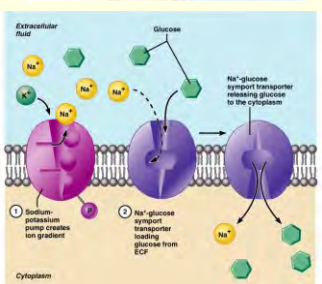


Active Transport

- Is transport of molecules against a concentration gradient and ATP is required
  - Primary
  - Co-transport
- **Example:**
  - Ca^{2+} Pump
  - Na^+/K^+ Pump - Uses ATP to move 3 Na^+ out and 2 K^+ in against their gradients



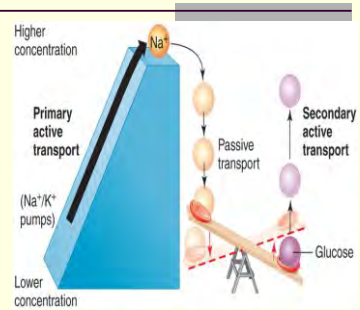




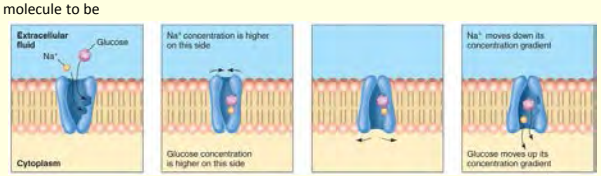
Adopted from Fox 2009

Secondary Active Transport

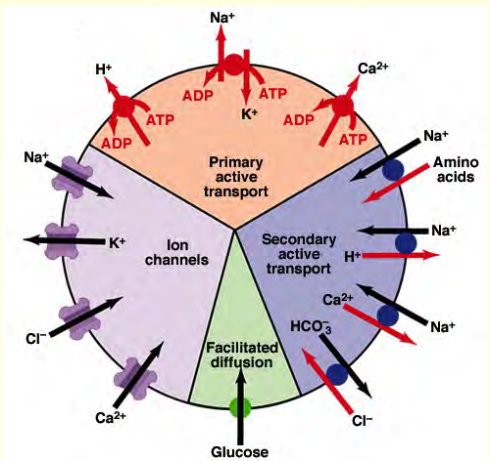
- Requires ATP to first move Na^+ uphill to create a gradient
  - Co-transport (symport) is secondary transport in same direction as Na^+
  - Counter-transport (antiport) moves molecule in opposite direction to Na^+
- Secondary active transport then uses energy from "downhill" movement of Na^+ to drive "uphill" transport of another molecule
  - One ion moves down its concentration gradient causing another ion/molecule to move against its concentration gradient
  - Ion acts as allosteric modulator - changes affinity of carrier for molecule to be transported



Adopted from Silverthorn 2010

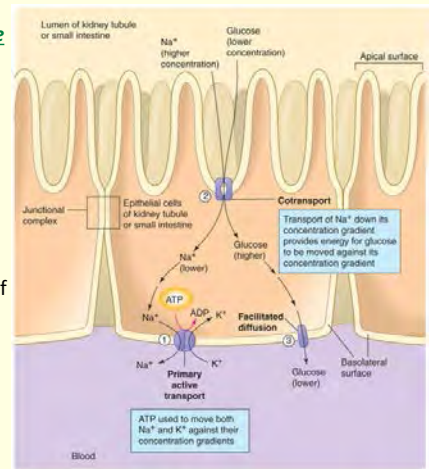


Transport Pathways Across Biological Membranes



Transport Across Epithelial Cells

- Epithelial transport is ***transcellular*** and uses ***membrane integral proteins***
- **Absorption** is transport of digestion products across intestinal epithelium into blood
- **Reabsorption** transports compounds out of urinary filtrate back into blood
- **Transcellular transport** moves material from one side to other of epithelial cells
- **Paracellular transport** moves material through tiny spaces between epithelial cells
  - Transport between cells is limited by junctional complexes that connect adjacent epithelial cells



Adopted from Silverthorn 2010

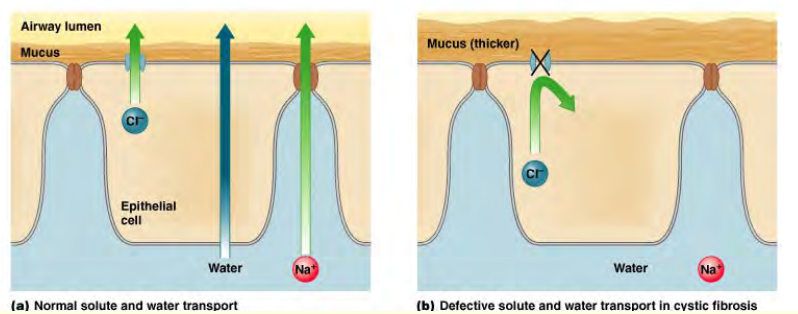
Severe diarrhea

- Treatment by Oral Rehydratation Therapy (ORT) – balance salt solution with glucose
- *Why is it working??*
- **EDEMA** – excessive accumulation of water in the tissues

Cystic fibrosis – abnormal CFTR

CFTR = Cystic fibrosis transmembrane conductance regulator

The predicted protein has 1,480 amino acids with a molecular mass of 168,138 Da. Mutations in the CFTR gene (most studied **delF508**)have been found to cause cystic fibrosis (CF) and congenital bilateral aplasia of the vas deferens.



(a) Normal solute and water transport

(b) Defective solute and water transport in cystic fibrosis

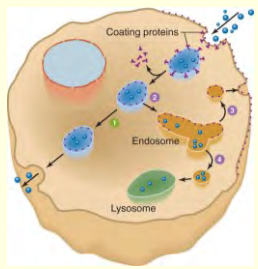
Adopted from Fox 2009

Bulk Transport -Exocytosis/Endocytosis

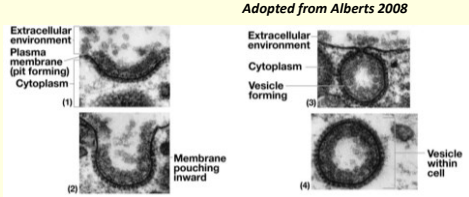
- Phagocytosis
- Pinocytosis
- Receptor-mediated endocytosis
- Potocytosis
- Transcytosis

Endocytosis – uptake of compounds generally too large to enter the cell via diffusion or active transport

- **Alternative functions of endocytosis:**
  - Transcellular transport
  - Endosomal processing
  - Recycling the membrane
  - Destroying engulfed materials

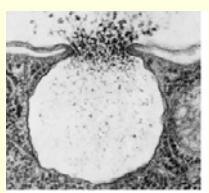


Adopted from Alberts 2008

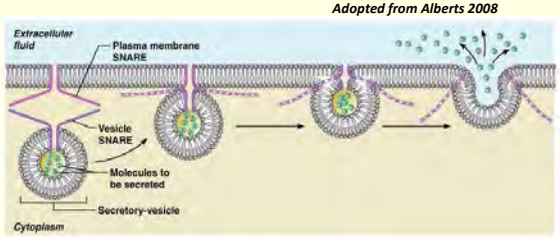


Exocytosis

- Stimulus for exocytosis is typically a cell-surface signal
- Exocytosis:
  - hormone secretion
  - neurotransmitter release
  - mucus secretion
  - waste ejection

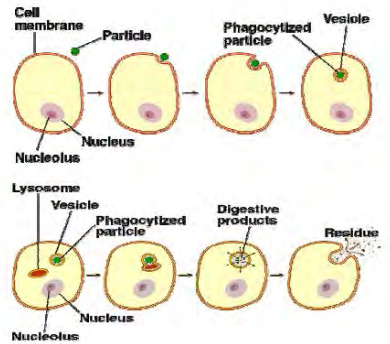


Adopted from Alberts 2008

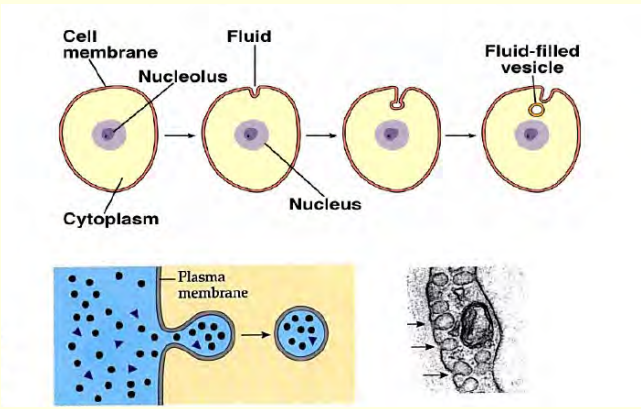


Exocytosis/Endocytosis

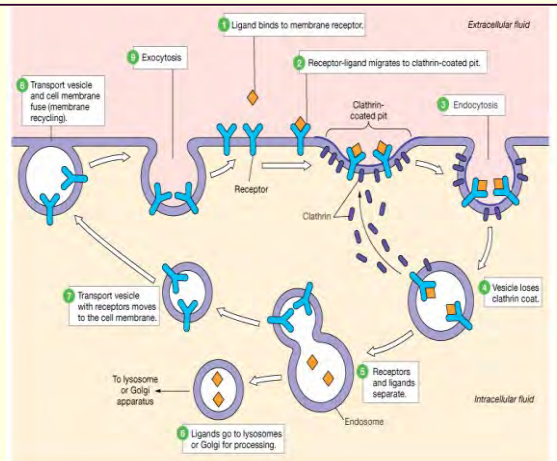
Alveolar macrophage phagocytosis of E. coli on the outer surface of a blood vessel (lung pleural cavity).



Pinocytosis - Cell intakes extracellular fluid nonspecifically (pino – drink). Requires energy.



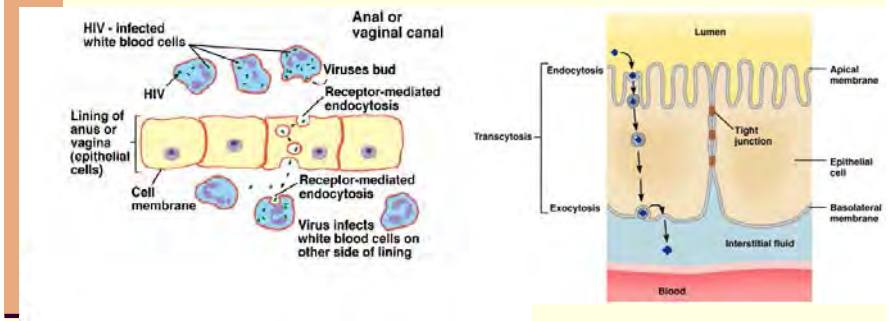
Receptor mediated endocytosis - Specific molecules in extracellular fluid bind to membrane bound receptors, triggering uptake of vesicles



Adopted from Silverthorn 2010

Transcytosis – Combines endo- and exo-cytosis.

Transfer of molecules across epithelial layers.

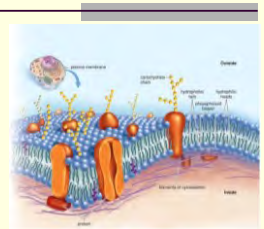


Membrane Potential

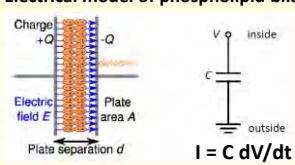
Plasma Membrane Electrical Model

- **Conductance** ***g = 1/R***
- **Capacitance** ***C = Q/V*** **-** because the membrane is so thin (≈ $6\times10^{-9}m$), we don't need much voltage to separate the charges and therefore the membrane capacitance is quite high; per unit area, it is

$$c = C/S \approx 10^{-2} Fm^{-2}$$



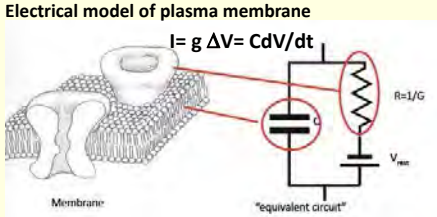
Electrical model of phospholipid bilayer



I = C dV/dt

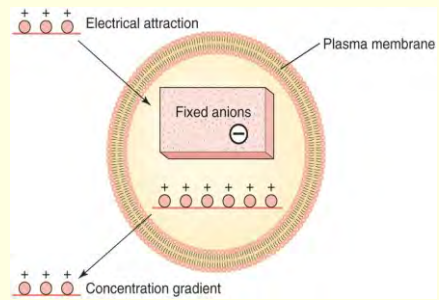
Electrical model of plasma membrane

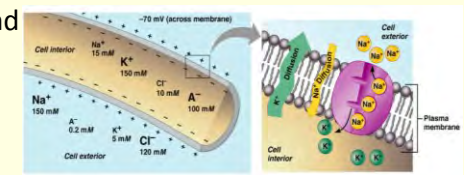
I= g ΔV= CdV/dt



Membrane Potential

- Is difference in charge across cell membranes
- Results in part from presence of large anions being trapped inside cell
  - Diffusable cations such as K^+ are attracted into cell by anions
- Na^+ is not permeable and is actively transported out





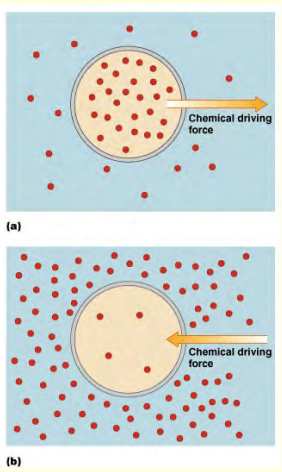
Adopted from Fox 2009

Chemical driving force

- **Concentration gradient = ΔC**
- **From high to low concentration**
  - **Down gradient**
  - **Spontaneous**
- **From low to high**
  - **Up gradient**
  - **Requires energy**

Magnitude of chemical driving force proportional to concentration gradient

Van'Hoff eq.: $\Delta G = RT \ln [X]_o/[X]_i$

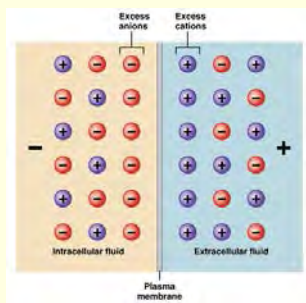


Adopted from Fox 2009

Electrical driving force

- Electrical driving force on charged molecules due to a membrane potential
- Separation of charge is a source of potential energy

$\Delta G = zFE$



Composition of Extracellular and Intracellular Fluids

	Extracellular [mM]	Intracellular [mM]
Na^+	150	15
K^+	5	150
Ca^{2+}	1	0.0001
Mg^{2+}	1.5	12
Cl^-	110	10
HCO_3^-	24	10
P_i	2	40
Amino acids	2	8
Glucose	5.6	1
ATP	0	4
Proteins	0.2	4

Summary of Chemical and Electrical Driving Forces

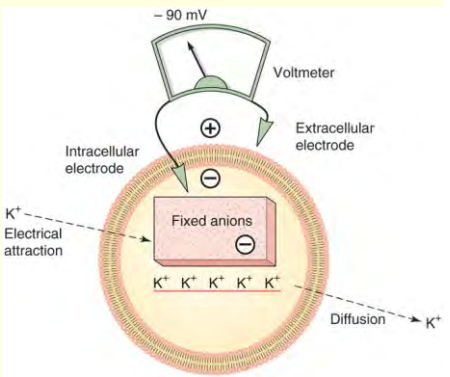
- **Chemical force = concentration gradient**
  - **direction: high → low**
- **Electrical force = charge separation**
  - **direction:**
    - **opposites attract**
    - **likes repel**

Electrochemical driving force:

$$\Delta G = RT \ln [X]_o/[X]_i + zFE$$

Equilibrium Potential

- Describes voltage across cell membrane in the situation when only one ion could diffuse
- If membrane permeable only to K^+, it would diffuse until it reaches its equilibrium potential (E_k)
  - K^+ is attracted inside by trapped anions but also driven out by its concentration gradient
  - At K^+ equilibrium, electrical and diffusion forces are = and opposite
  - Inside of cell has a negative charge of about E_K= -90mV



Adopted from Fox 2009

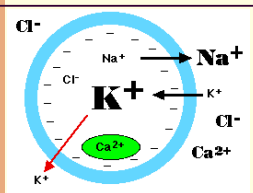
Equilibrium Potential

- **$\Delta G = RT \ln [X]_o/[X]_i + zFE$**
- $\Delta G = 0$ at equilibrium potential
  - $\Delta G<0$ => movement down a concentration gradient
  - $\Delta G>0$ => movement down a concentration gradient
- **$zFE_{eq} = RT \ln [X]_o/[X]_i$** ⇒ **$E_{eq} = (RT/zF) \ln [X]_o/[X]_i$**
- **$RT/F = 61$ mV (at 37°C)** ⇒ **$E_{eq} = (61/z) \log [X]_o/[X]_i$**

Nernst equation

$$E_{eq} = (61/z) \log [X]_o/[X]_i$$

Summary of Membrane Potential



Ion distribution in the cell

Ion	Concentration outside (in mM)	Concentration inside (in mM)	Ratio Out : In
K^+	5	100	1 : 20
Na^+	150	15	10 : 1
Ca^{2+}	2	0.0002	10,000 : 1
Cl^-	150	13	11.5 : 1

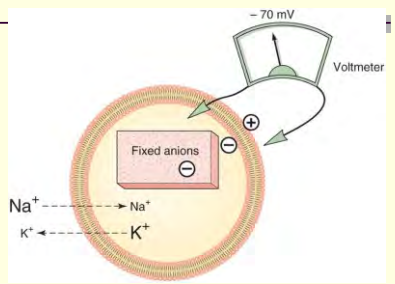
The Goldman-Hodgkin-Katz equation

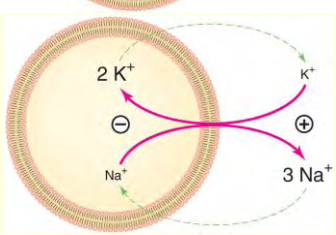
$$V_m = \frac{RT}{F} \ln \left(\frac{p_K[K^+]_o + p_{Na}[Na^+]_o + p_{Cl}[Cl^-]_i}{p_K[K^+]_i + p_{Na}[Na^+]_i + p_{Cl}[Cl^-]_o} \right)$$

- V_m is the membrane potential
- R is the universal gas constant (8.314 $J.K^{-1}.mol^{-1}$)
- T is the temperature in Kelvin (K = °C + 273.15)
- F is the Faraday's constant (96485 $C.mol^{-1}$)
- $p_{K,\ Na\ or\ Cl}$ is the membrane permeability for K^+, Na^+, or Cl^-, respectively. Normally, permeability values are reported as relative permeabilities with p_K having the reference value of one (because in most cells at rest p_K is larger than p_{Na} and p_{Cl}). For a typical neuron at rest, $p_K : p_{Na} : p_{Cl} = 1 : 0.05 : 0.45$. Note that because relative permeability values are reported, permeability values are unitless.

Resting Membrane Potential (RMP)

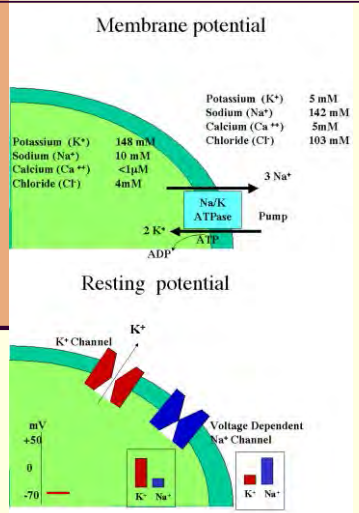
- Is membrane voltage of cell at rest
- RMP of most cells is –65 to –85 mV
- RMP depends on concentrations of ions inside and out and on permeability of each ion
  - Affected most by K^+ because it is most permeable
  - Some Na^+ diffuses in so RMP is less negative than E_{K+}
- **Role of Na/K Pump** - Because 3 Na+ are pumped out for every 2 K+ taken in, pump is electrogenic
  - It adds about
  - –3mV to RMP

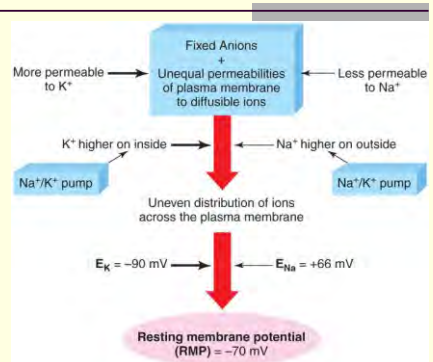




Adopted from Fox 2009

Summary of Processes that Affect the Resting Membrane Potential



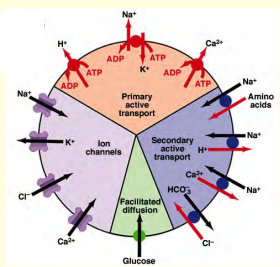


The Goldman-Hodgkin-Katz equation

$$V_m = \frac{RT}{F} \ln\left(\frac{p_K[K^+]_o + p_{Na}[Na^+]_o + p_{Cl}[Cl^-]_i}{p_K[K^+]_i + p_{Na}[Na^+]_i + p_{Cl}[Cl^-]_o}\right)$$

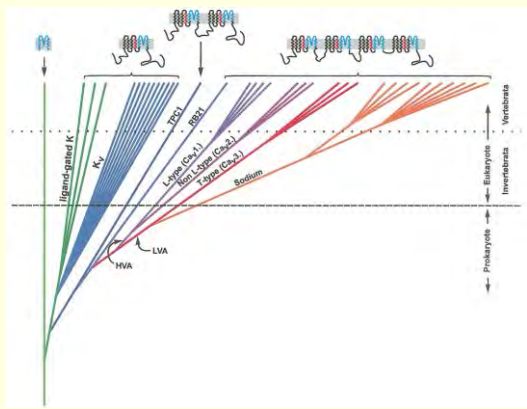
Properties of Ion Channels

- Integral proteins of plasma membrane - membrane spanning - several transmembrane domains in the channel protein
- Allow rapid ion movement (flow 10^8/s) - diffusion of ions is passive, down electrochemical gradients
- Selective - Selective to either anions or cations, or to specific cations (eg. K+, Na+, etc.)
- Gated - Open and closed states, activated by voltage, ligand binding or physical changes
- Modulated - Modulated by auxiliary subunits, G proteins or neuromodulators



Phylogeny of ion channels

- The model predicts that voltage-gated ion channels evolved over time from a prokaryote 2-TM channel.
- Following the addition of four more domains, an early, ligand-gated, 6-TM protein gave rise to the voltage-gated K family Kv and then, following two rounds of gene duplication, formed a four-domain 6-TM channel.
- Early four-domain channels were likely non-selective, but some are assumed to have developed calcium selectivity, giving rise to LVA and HVA calcium channels. Sodium channels are thought to have evolved from LVA
- channels.

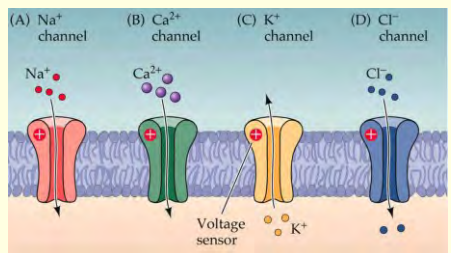


A hypothetical phylogeny of voltage-gated ion channels.
Adopted from Anderson & Greenberg (2001) Comp.Biochem.Physiol. Part B 129: 17-18

Ion channels

- **NON-GATED CHANNELS -** plasma membrane has many more K^+ non-gated channels than Na^+non-gated channels – thus membrane permeability to $K^+$is higher
- **GATED CHANNELS•**
  - **Voltage-gated**: Na^+, K^+, Ca^{2+}, Cl^--many subtypes within each category **– voltage dependent**
  - **Ligand-gated**: nAChR, GABAAR, GluR(glutamate), glyR(glycine) -many subtypes within each category – **ligand dependent ION**
- Best source for ion channel knowledge – „Bertil Hille: Ion channels of excitable membranes.“

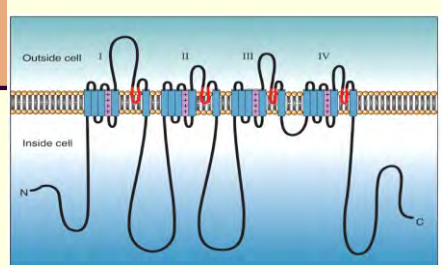
Voltage-dependent ion channels

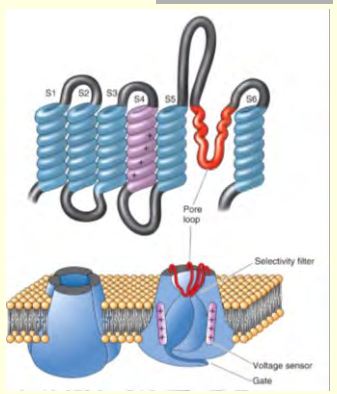


- Open and close in response to **voltage change**
- Found in excitable (cardiac, skeletal, and neuronal) and non-excitable cells
- **Voltage change** causes membrane potential difference that can lead to depolarization of the cell
- Play a role in an **action potential**
- Share the same basic structural features.

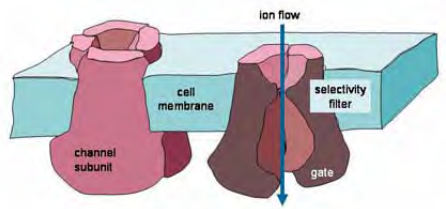
Structure of voltage-gated (VG) channels

- 4 homologous subunits come together to function as a channel
- each with 6 TM segments (S)
- **S4**: ***voltage sensor***
- **S5, S6**: form pore
- ***P loop***(between S5 and S6): ion selectivity
- ***Inactivation gate***





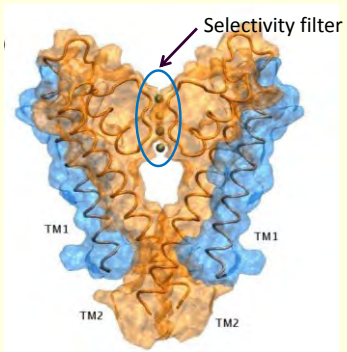
Functional domains of VG ion Channels



- **1.Ion selectivity filter**-selective to Na^+, K^+ etc.
- **2.Voltage sensor**-positively charged amino acids, which responds to changes in the membrane potential
- **3.Inactivation Gate**-opens and closes a channel; controlled by a voltage sensor

Selectivity filter

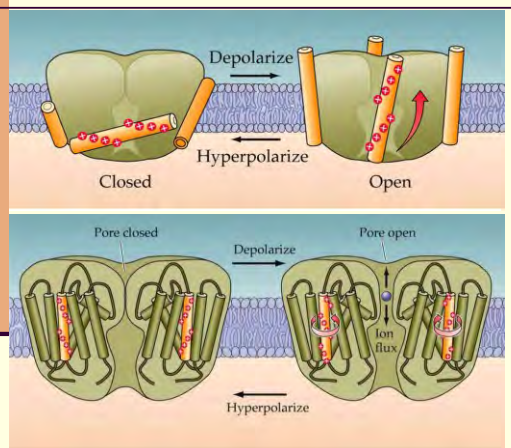
- The selectivity filter is the name for the narrowest region of an ion channel pore, which interacts with the permeating ions and ensures that ions pass through the channel selectively.
- Because the selectivity filter is what defines the nature of a given ion channel, this region is highly conserved both in sequence and in structure across a given type of ion channel



A side view of the KcsA K^+ channel.

Adopted from Roderick Mackinnon. Potassium channels and the atomic basis of selective ion conduction (nobel lecture). Angew. Chem. Int. Ed., 43:4265–4277, 2004.

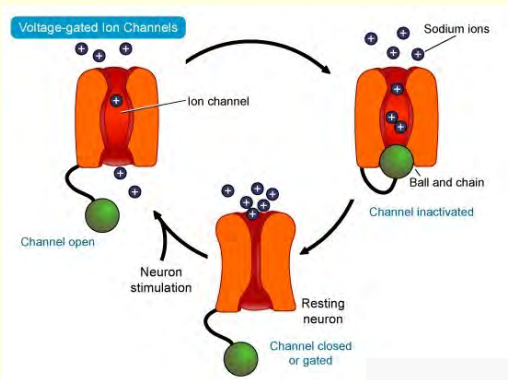
Voltage sensor

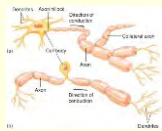


- **Voltage sensor = S4 TM**
- Consist of **positively charges amino acid** residues (arginine and lysine)
- **At rest** the positively charged AA residues are attracted to the inner residues of S2 and S3
- **When the membrane is more positive**(during AP), **S4** is electrostatically repelled
  - **Rotates** from a slanted, non-permissive position to a more upright position which permits ion conductance through the channel

Inactivation gate

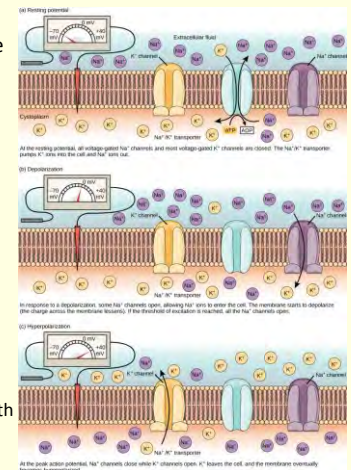
- The typical voltage-gated channel opens on depolarization and closes rapidly on repolarization or, more slowly, on sustained depolarization. The latter process is termed inactivation and leaves the channel refractory for sometime after repolarization.
- **Two types of inactivation:**
- 1. **Fast** the "classical" inactivation (milliseconds) range
- 2. **Slow** inactivation (up to seconds)
- ***These two types of inactivation have different mechanisms*** located in different parts of the channel molecule:
- The **fast** inactivation at the cytoplasmic pore opening which can be closed by a hinged lid.
- The **slow** inactivation in other parts involving conformational changes of the pore.





Excitable Cells

- Electrically excitable cells – respond to signals through changes in their membrane potential – neurons and muscle cells
- Changes in membrane potential arise from activation of ION CHANNELS
- If the membrane becomes more positive inside, the cell has "DEPOLARIZED" – it can happen by
  - Na^+ moves in or decrease of K^+ efflux
  - Depolarization, in general, correlates with increased excitability
- If the membrane becomes more negative inside, the cell has "HYPERPOLARIZED"
  - K^+ moves out or Cl^- moves in
  - Hyperpolarization, in general, correlates with decreased excitability



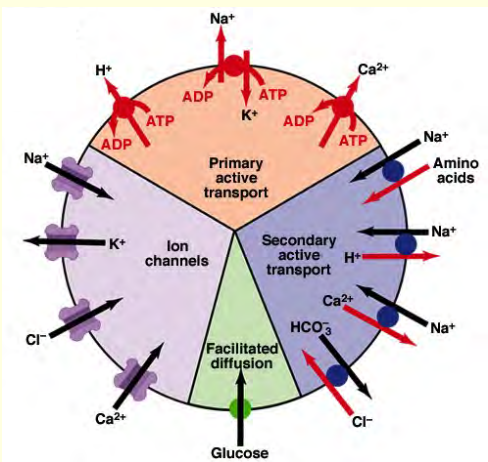
Adopted from Fox 2009

Membrane Potential and Excitable Cells

Membrane Potential and Excitable Cells

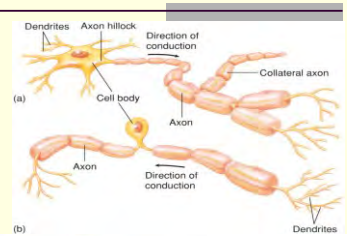
- Membrane potential
- Ion channels
- Action potential
- Cell signaling

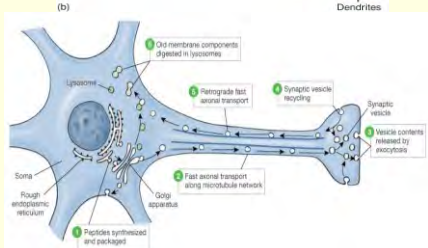
Summary of Transport Pathways Across Cell Membranes



Neurons

- ***Function:*** Gather and transmit information by:
  - Responding to stimuli
  - Producing and sending electrochemical impulses
  - Releasing chemical messages
- ***Structure:*** Have a cell body, dendrites and axon
  - **Cell body** is the nutritional center and makes macromolecules
    - site of energy generation and synthesis
    - contains the nucleus
    - electrical depolarizations
  - **Dendrites** receive information, convey it to cell body
  - **Axons** conduct impulses away from cell body
- **Axons** - Long axon length necessitates special transport systems:
  - **Axoplasmic flow** moves soluble compounds toward nerve endings
    - Via rhythmic contractions of axon
  - **Axoplasmic transport**
    - Fast axonal transport to the terminal
    - Retrograde to cell body



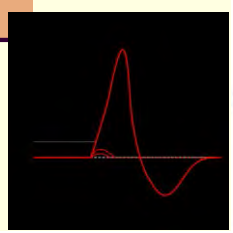


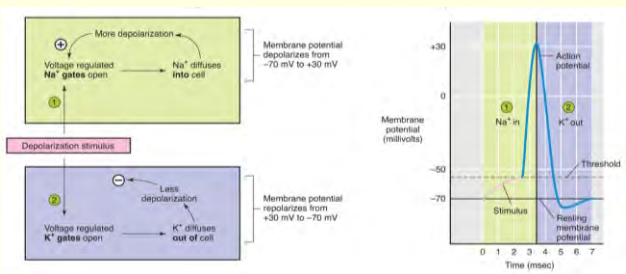
Adopted from Silverthorn 2010

The nerve impulse: Action Potential (AP)

- Action potential – rapid change in the axon membrane that allows a nerve impulse to occur
- Is a wave of MP change that sweeps along the axon from soma to synapse
- Wave is formed by rapid depolarization of the membrane by Na^+ influx; followed by rapid repolarization by K^+ efflux

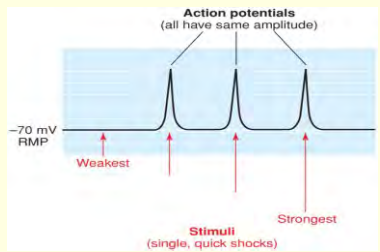
Adopted from Fox 2009

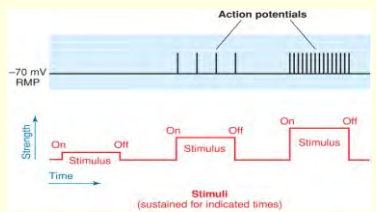




APs Are All-or-None

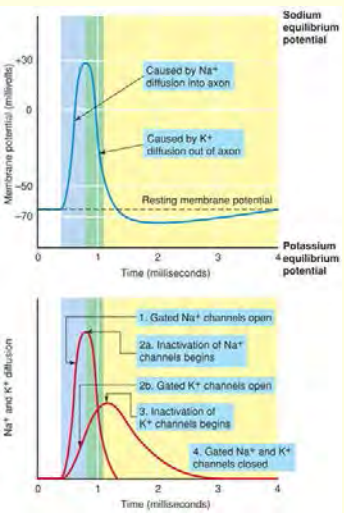
- When MP reaches threshold an AP is irreversibly fired
  - Because positive feedback opens more and more Na^+ channels
  - Shortly after opening, Na^+ channels close and become inactivated until repolarization
- **How Stimulus Intensity is Coded** - Increased stimulus intensity causes more APs to be fired
  - Size of APs remains constant





Mechanism of Action Potential

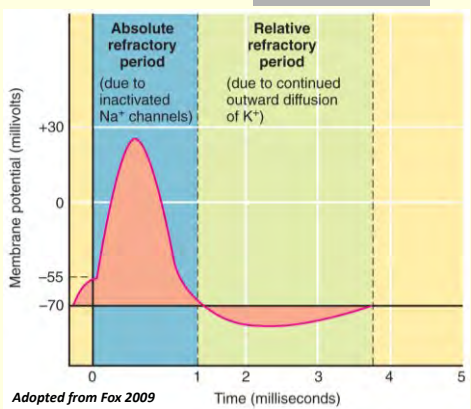
- **Depolarization:**
  - At threshold, VG Na^+ channels open
  - Na+ driven inward by its electrochemical gradient
  - This adds to depolarization, opens more channels
    - Termed a positive feedback loop
  - Causes a rapid change in MP from –70 to +30 mV
- **Repolarization:**
  - VG Na^+ channels close; VG K^+ channels open
  - Electrochemical gradient drives K^+ outward
  - Repolarizes axon back to RMP
- Depolarization and repolarization occur via diffusion
  - Do not require active transport
  - After an AP, Na^+/K^+ pump extrudes Na^+, recovers K^+
- Resting potential is restored by moving potassium inside and sodium outside



Adopted from Fox 2009

Refractory Periods

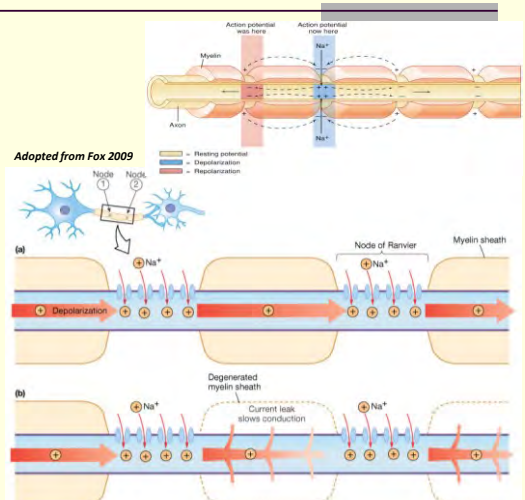
- Absolute refractory period:
  - Axon membrane is incapable of producing another AP.
    - because Na^+ channels are inactivated
- Relative refractory period:
  - VG ion channel shape alters at the molecular level.
  - VG K^+ channels are open - making it harder to depolarize to threshold
  - Axon membrane can produce another action potential, but requires stronger stimulus.



Adopted from Fox 2009

Conduction of action potentials in myelinated axons

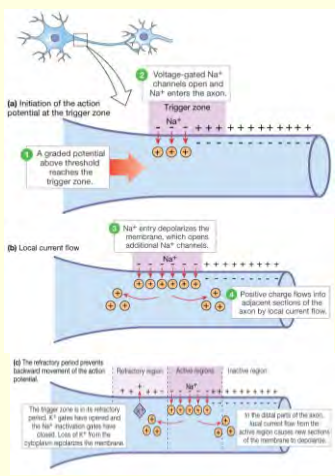
- Myelin prevents movement of Na+ and K+ through the membrane.
- Interruption in myelin (Nodes of Ranvier) contain VG Na+ and K+ channels.
- AP occurs only at the nodes.
  - AP at 1 node depolarizes membrane to reach threshold at next node.
- **Saltatory conduction** (leaps).
  - Fast rate of conduction (**up to 100m/s**).



Adopted from Fox 2009

Conduction of action potentials in unmyelinated axons

- Cable spread of depolarization with influx of Na^+ depolarizes the adjacent region membrane, propagating the AP.
- Conduction rate is slow.
  - AP must be produced at every fraction of micrometer (**1m/s**).
- Occurs in one direction; previous region is in its refractory period.

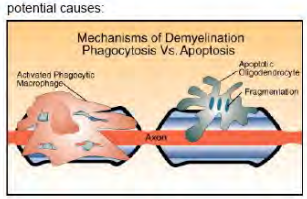


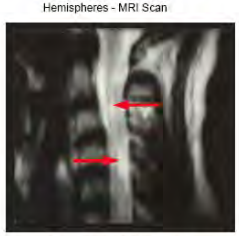
Adopted from Silverthorn 2010

Multiple Sclerosis

Demyelination in Cerebral Hemispheres - MRI Scan

potential causes:





Demyelination in the Cervical Spinal Cord - MRI Scan

Common Symptoms include:

- A "pins and needles" prickling sensation most often in your toes or fingers - like your foot or hand fell asleep
- Numbness - again most often in your toes or fingers
- Problems with speech and swallowing
- Eye trouble - seeing double, blurry vision, or uncontrolled eye movements
- Slight memory problems
- Tremors in your arms, wrists, and hands - particularly when you try to pick something up or write
- Loss of balance and poor coordination
- Gait difficulties
- Extreme weakness
- Abnormal fatigue
- Partial or complete paralysis
- Bowel and bladder dysfunction
- Sexual function problems

Comparison of Conduction Velocities of Nerves

Diameter (μm)	Conduction Velocity (m/sec)	Examples of Functions Served
12–22	70–120	Sensory: muscle position
5–13	30–90	Somatic motor fibers
3–8	15–40	Sensory: touch, pressure
1–5	12–30	Sensory: pain, temperature
1–3	3–15	Autonomic fibers to ganglia
0.3–1.3	0.7–2.2	Autonomic fibers to smooth and cardiac muscles

Factors affecting generation of AP

❖ Factors that can alter electrical signaling

TOXINS

Voltage-gated Na^+ channels

Block channel function:

- Tetrodotoxin – from puffer fish
- Saxitoxin – from dinoflagellates
- Conotoxin – from marine snail, *Conus*
- …many local anesthetics (procaine, cocaine)

→ Causes failure to generate AP's (no Na^+ influx)
Change in potential becomes a graded potential that decays away rapidly, likely unable to cause transmitter release from the terminal

Block inactivation of the channel:

- Sea anenome toxins
- Scorpion toxins
- Stony coral toxin

→ Na^+ channels cannot inactivate; failure to elicit normal AP's; signal failure

Voltage-gated K^+ channels

Block channel function:

- Tetraethylammonium
- Phencyclidine (PCP)
- Margatoxin
- Gaboon viper venom

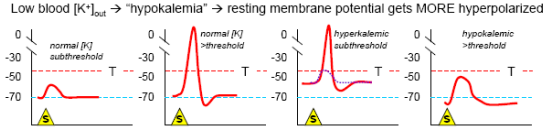
→ Cells can't repolarize properly; loss of membrane potential, AP failure

CHANGES in PLASMA [K^+]

$[K^+]_{out}$ = major determinant of resting potential (~3.5 – 5 mM); tightly regulated.

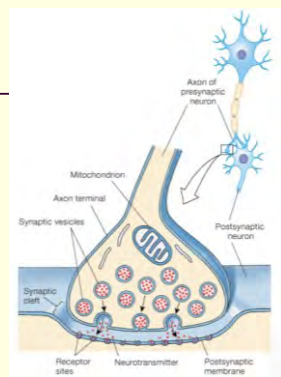
High blood $[K^+]_{out}$ → "hyperkalemia" → resting membrane potential gets MORE depolarized

Low blood $[K^+]_{out}$ → "hypokalemia" → resting membrane potential gets MORE hyperpolarized



Synapse

- Functional connection between a neuron and another neuron or effector cell.
- Transmission in one direction only.
- Axon of first (presynaptic) to second (postsynaptic) neuron.
- Synaptic transmission is through a electrically or chemically gated channel.
  - Electrical synapses are rare in NS
- Presynaptic terminal (bouton) releases a neurotransmitter (NT).



Adopted from Fox 2009

Type of Synapse	Distance between pre- & post-synaptic membranes	Cytoplasmic continuity between pre- & post-synaptic membranes	Structural components	Agent of transmission	Synaptic delay	Direction of transmission
Electrical	3.5 nm	Yes	Gap-junctional channels	Ion current	Virtually none	Usually bidirectional
Chemical	20-40 nm	No	Presynaptic vesicles & active zones; postsynaptic receptors	Chemical transmitter	Significant; at least 0.3 ms, usually 1-5 ms or longer	Uni-directional

Chemical Synapses

Chemical Synapses

Most common mode of intercellular communication

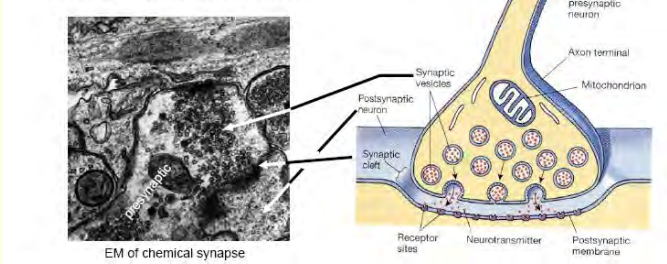
excitation of presynaptic cell

release of chemical transmitter

diffuses across synaptic cleft

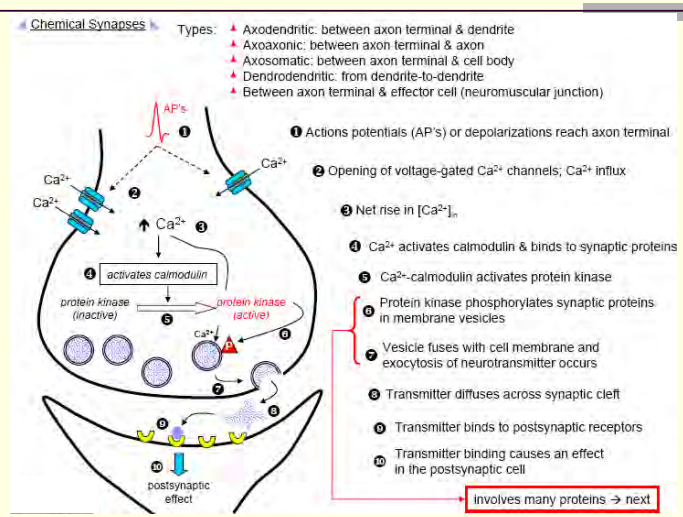
binds to specific receptors on postsynaptic membrane

causes a change in postsynaptic cell physiology

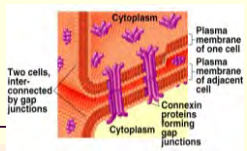


EM of chemical synapse

Synaptic Transmission in Chemical Synapse



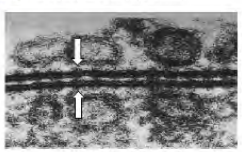
Electrical Synapses



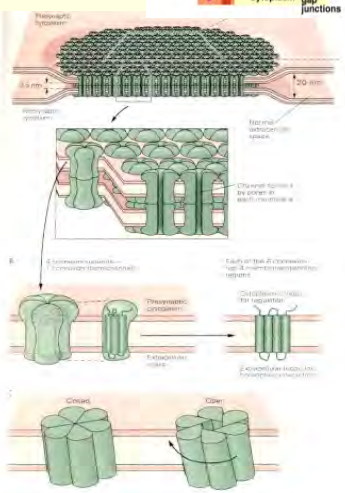
Electrical Synapses (Gap Junctions)

Structure:

- Gap-junctional channel is pair of hemichannels, each hemichannel, called a **connexon**, resides in one of the two adjoining cells.
- Each connexon is made of 6 subunits, called **connexins**.
- Each connexin has 4 membrane spanning units whose extracellular loops form the site of interactions between connexons in coupled cells.
- When paired, cytoplasms of the two coupled cells are contiguous.



EM showing gap junction proteins



Electrical Synapses

Electrical Synapses (Gap Junctions)

Roles:

- Useful when speed of response is advantageous
- Useful for functionally connecting large numbers of cells [1]
  - synchronize firing of action potentials
- May contribute to sharing of *metabolic signals* between cells (up to 1000 MW) [2]
- Particularly important for glial cell function

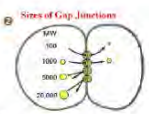
Schwann cells: successive layers of myelin connected by gap junctions. Thought to be necessary for passage of signals, nutrients, metabolites

Charcot-Marie-Tooth disease:
"Hereditary Motor and Sensory Neuropathy"
—a disease of demyelination, results from a single mutation in one connexin gene. This prevents Schwann cells from forming functional gap junctions.

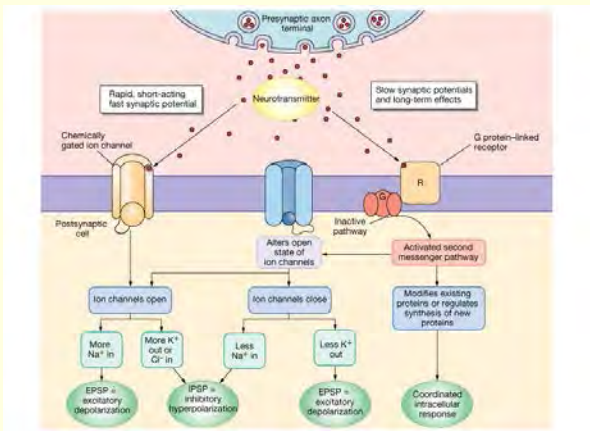
The neuropathy of CMT affects both motor and sensory nerves. A typical feature includes weakness of the foot and lower leg muscles, which may result in foot drop and a high-stepped gait with frequent tripping or falls. Foot deformities [3], such as high arches and hammertoes (a condition in which the middle joint of a toe bends upwards) are also characteristic due to weakness of the small muscles in the feet. In addition, the lower legs may take on an "inverted champagne bottle" appearance due to the loss of muscle bulk. Later in the disease, weakness and muscle atrophy may occur in the hands, resulting in difficulty with fine motor skills. Although sensory nerves are also involved, patients rarely notice significant numbness or pain.

treatment ?

coupling on neurons revealed by small molecular weight dye injected into a single cell



Multiple Receptors modify signal



Adopted from Fox 2009

Pathologies

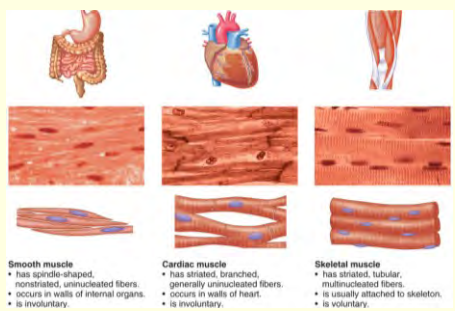
- Synaptic transmission
  - Drugs in ECF
  - Disorders of ion balance
  - Too much/too little NT release
  - Examples: Parkinson's, schizophrenia, epilepsy, depression
- Nerve injury
  - Limited regrowth
  - Parallel nerves help some

Excitable cells - Muscle

- Basic anatomy
- Muscle fiber anatomy
  - contractile proteins
  - accessory proteins
- Definition of muscle movements
- Excitable events in muscle
  - Action potential
  - Excitation-contraction coupling

Muscle Similarities

- Skeletal and smooth muscle cells are elongated and are called muscle fibers
- Muscle contraction depends on two kinds of myofilaments – actin and myosin
- Muscle terminology is similar
  - Sarcolemma – muscle plasma membrane
  - Sarcoplasm – cytoplasm of a muscle cell
  - Prefixes – myo, mys, and sarco all refer to muscle



Functional Characteristics of Muscle Tissue

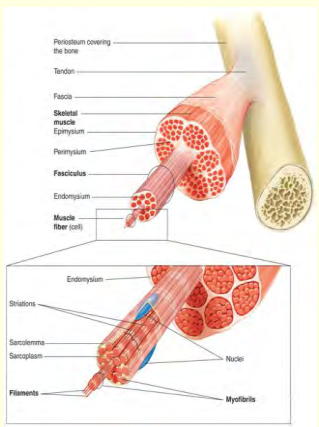
- Excitability– the ability to receive and respond to stimuli
- Contractility – the ability to shorten forcibly
- Extensibility – the ability to be stretched or extended
- Elasticity – the ability to recoil and resume the original resting length

Skeletal Muscle Tissue

- Packaged in skeletal muscles that attach to and cover the bony skeleton
- Has obvious stripes called striations
- Is controlled voluntarily (i.e., by conscious control)
- Contracts rapidly but tires easily
- Is responsible for overall body motility

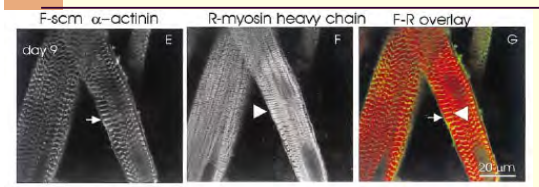
Muscle Tone

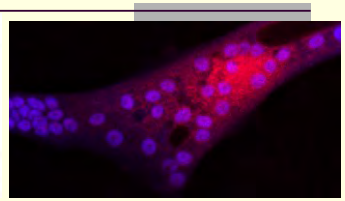
- Low level of contractile activity in relaxed muscles that keep muscles healthy and ready to react to stimulation
- Muscle tone is extremely important in reinforcing the shoulder and knee joints and the arches of the foot.



Adopted from Fox 2009

Skeletal Muscle Tissue

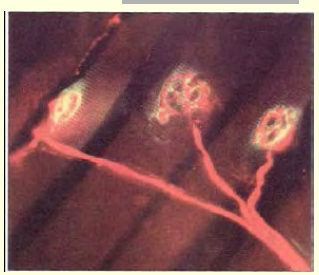




- Packaged in skeletal muscles that attach to and cover the bony skeleton
- Has obvious stripes called striations
- Muscle fibers are multinucleated
- Is controlled voluntarily (i.e., by conscious control)
- Contracts rapidly but tires easily
- Is responsible for overall body motility
- Is extremely adaptable and can exert forces ranging from a fraction of a kg (few grams) to over 20 kg.

Skeletal Muscle: Nerve and Blood Supply

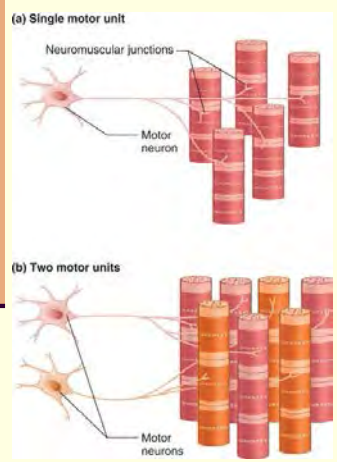
- Each muscle is served by one nerve, an artery, and one or more veins
- Each skeletal muscle fiber is supplied with a nerve ending that controls contraction
- Contracting fibers require continuous delivery of oxygen and nutrients via arteries
- Wastes must be removed via veins



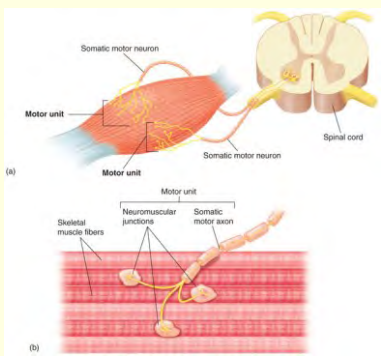
Motor unit

- When a motor neuron is activated, all muscle fibers in its motor unit contract
- Number of muscle fibers in motor unit varies according to degree of fine control capability of the muscle
  - Innervation ratio is number of motor neurons to number of muscle fibers
    - Vary from 1:100 to 1:2000
  - Fine control occurs when motor units are small, i.e. 1 motor neuron innervates small # of fibers
- Since individual motor units fire "all-or-none," how do skeletal muscles perform smooth movements?
  - Recruitment is used:
    - Brain estimates number of motor units required and stimulates them to contract
    - It keeps recruiting more units until desired movement is accomplished in smooth fashion
    - More and larger motor units are activated to produce greater strength

Motor unit = Motor neuron + muscle fibers that are innervated by neuron



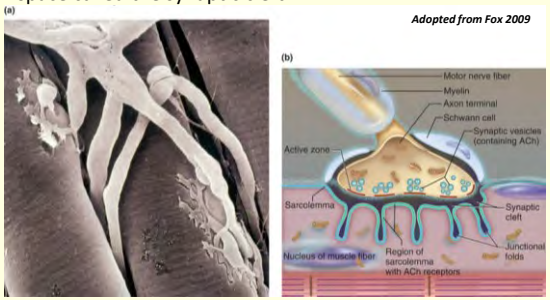
One fiber is innervated by only one neuron!!!



Adopted from Fox 2009

Neuromuscular Junction – Site of Action Potential

- The neuromuscular junction is formed from:
  - Axonal endings, which have small membranous sacs (synaptic vesicles) that contain the neurotransmitter acetylcholine (ACh)
  - The motor end plate of a muscle, which is a specific part of the sarcolemma that contains ACh receptors and helps form the neuromuscular junction
- Though exceedingly close, axonal ends and muscle fibers are always separated by a space called the synaptic cleft



Excitation-contraction

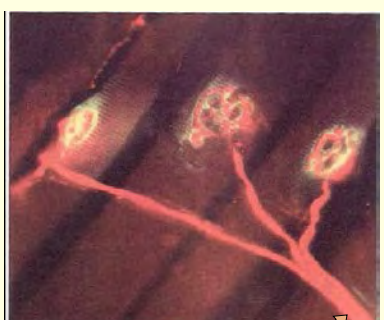
- Molecular mechanism for contraction
- Regulation of contraction
  - E-C coupling
  - Relaxation
- Muscle metabolism
- Fatigue
- Fiber types

Regulation of contraction

E-C coupling- link of events leading to contraction

What causes the initial rise in Ca^{2+}? (what initiates contraction?)

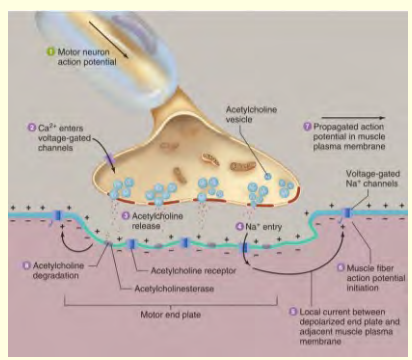
- central nervous system
- somatic motor neurons
- action potentials in neurons lead to release of acetylcholine
- acetylcholine depolarizes the muscle fibers
- depolarization leads to rise in $[Ca^{2+}]_{in}$ in sarcoplasm

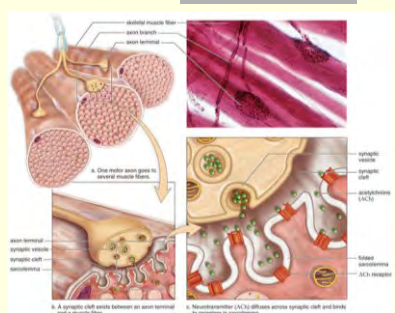


motor neuron

Steps in skeletal muscle contraction

1. Nerve impulses travel down motor neurons to a neuromuscular junction



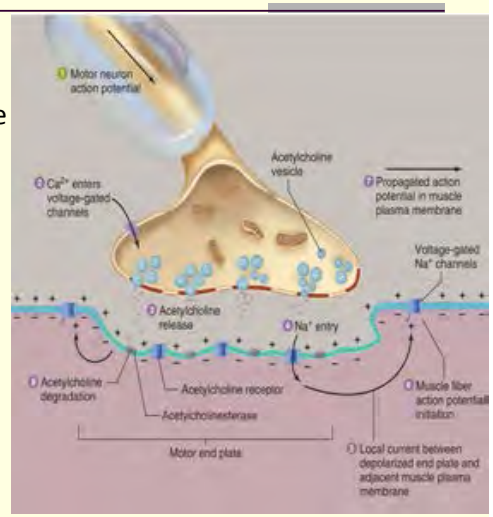


Adopted from Fox 2009

Steps in skeletal muscle contraction

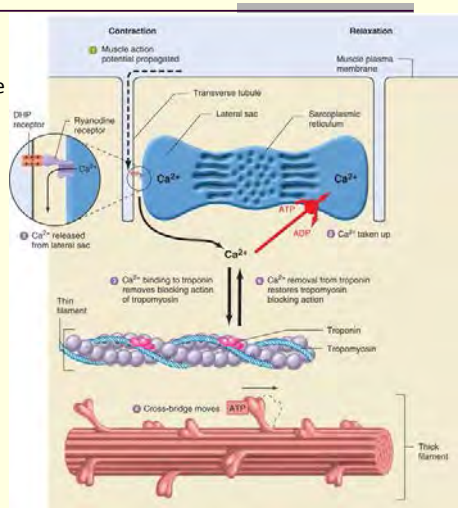
2. Acetylcholine (ACh) is released from the neurons and bind to the muscle fibers

Mechanism of action potential in skeletal muscle fiber is the same as in neuron.



Steps in skeletal muscle contraction

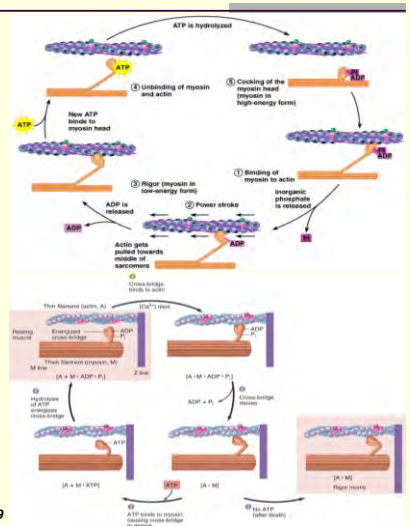
3. This binding evokes muscle action potential
4. AP triggers conformational change in Cav1.1 channel (DHP receptor) followed by release of Ca2+ from the sarcoplasmic reticulum
5. Released calcium combines with troponin, a molecule associated with actin
6. This causes the tropomyosin threads around actin to shift and expose myosin binding sites
7. Myosin heads bind to these sites forming cross-bridges
8. ATP bind to the myosin heads and is used as energy to pull the actin filaments towards the center of the sarcomere = contraction now occurs



Adopted from Silverthorn 2010

Visualizing the role of calcium and myosin in muscle contraction *Crossbridge Cycle*

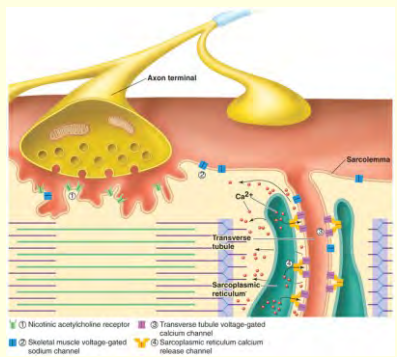
- ATP is needed to attach and detach the myosin heads from actin
- After death muscle cells continue to produce ATP through fermentation and muscle cells can continue to contract
- When ATP runs out some myosin heads are still attached and cannot unattach = rigor mortis
- Body temperature and rigor mortis helps to estimate the time of death



Adopted from Fox 2009

Muscle Relaxation

- Ca^{++} from SR diffuses to troponin to initiate crossbridge cycling and contraction
- When APs cease, muscle relaxes
  - Because Ca^{++} channels close and Ca^{++} is pumped back into SR
- Ca^{++} levels decrease because it is continually pumped back into the sarcoplasmic reticulum (SR - a calcium reservoir in muscle)



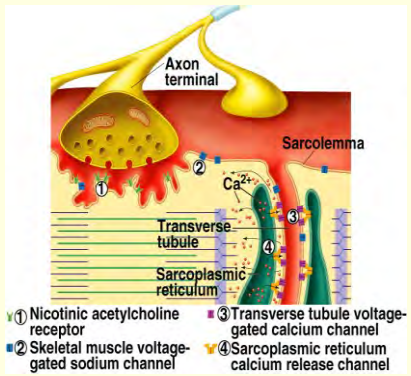
Adopted from Fox 2009

Clinical Connection

- *Myesthenia gravis* – decreased number of AchRs over time – autoimmune disease
- *Venoms and toxins* – target AchR
  - either blocking effect (curare, botulinum toxin, nerve gases) **flaccid paralysis**
  - activating effect (venom from rattlesnake and spiders) **paralytic spasm**

Clinical Connection – Genetic illnesses

- *Skeletal muscle Na^+ channel* – paramyotonia congenita, potassium aggressive myotonia, hypokalemic periodic paralysis
- *DHPR* – hypokalemic periodic paralysis, myotonia, malignant hypothermia
- *RyR1* - malignant hypothermia, central core disease



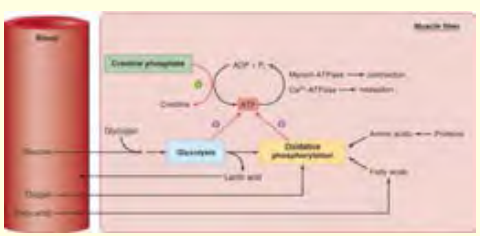
Adopted from Fox 2009

Muscle metabolism

- ATP is required at three stages:
  - **1** cross bridge formation
  - **2** pumping Ca^{2+}back into SR
  - **3** help restore Na^{+}/K^{+} gradient in muscle cell.

Amount of ATP in a muscle cell will only generate about 8 brief twitches

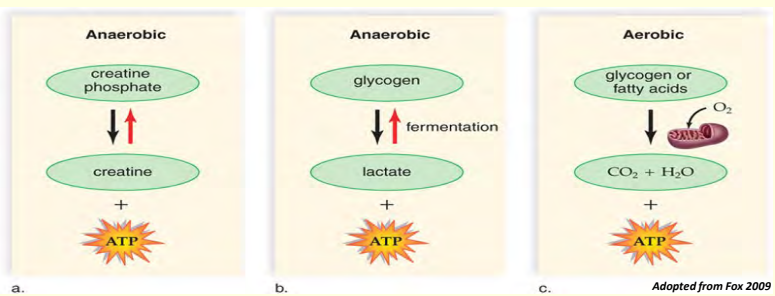
Muscles store creatine phosphate as a source of energy



Adopted from Silverthorn 2010

Sources of ATP for muscle contraction

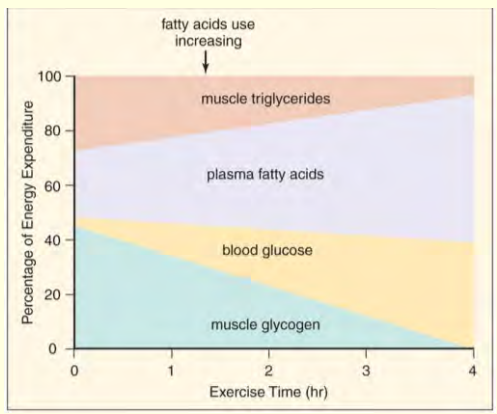
- Limited amounts of ATP are stored in muscle fibers
- Anaerobic respiration
  - Creatine phosphate pathway (CP) – fastest way to acquire ATP but only sustains a cell for seconds; builds up when a muscle is resting
  - Lactate pathway – fast-acting but results in lactate build up
- Cellular respiration (aerobic) – not an immediate source of ATP but the best long term source



Adopted from Fox 2009

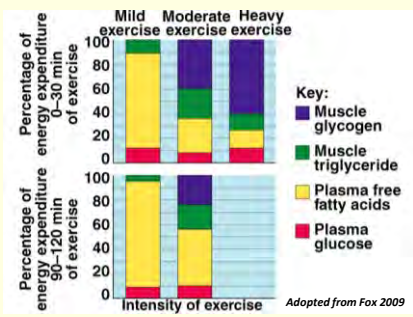
Fuel sources for muscle contraction

- Stored in the muscle:
  - Glycogen
  - Fat
- In the blood:
  - Glucose
  - Fatty acids



Muscle metabolism

- *Low intensity-to-moderate exercise:* enough O_2 to keep muscle producing ATP aerobically
- *High intensity exercise:* switch to anaerobic respiration (build up of lactic acid)
  - *develop an "oxygen debt": O_2 being used to generate ATP, not to convert lactic acid back into glucose. Post-exercise, must "repay this debt" by converting lactic acid built up back into glucose*
- **Note:** Lack of ATP almost never occurs in muscle (max utilization ~30% of possible ATP)



Fatigue

High intensity exercise over time → muscle loses ability to contract or to sustain a contraction "fatigue"

Possible causes of fatigue:

1. Changes in ionic composition of the muscle fiber after many contractions
   - Elevated intracellular Na^+
   - Depleted intracellular K^+
2. Depletion of muscle nutrients
3. Diminished neurotransmitter (ACh) output from presynaptic motor neuron (*unlikely*)
4. Excitation-contraction failure
   a. Extended submaximal exertion → depleted glycogen stores
      - **BUT:** research shows lack of ATP is rarely, if ever, a limiting factor
      - **So**, glycogen depletion must be affecting another aspect of contraction (**?** Ca release from SR)

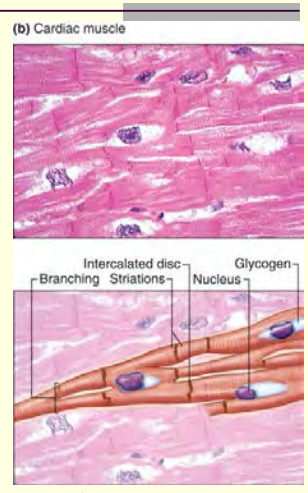
   b. Short-duration, maximal exertion → increased production of inorganic phosphate (P_i)
      - Elevated P_i slows P_i release from myosin, altering the power stroke. *(Recall, release of P_i initiates the power stroke)*

   c. Elevated extracellular K^+
      - Each action potential → K^+ efflux → increase in $[K^+]$ extracellularly → → decrease Ca^{2+} release from SR

   d. Buildup of lactic acid → lowered pH in muscle tissue, which is associated with decrease in ability to respond → **"central fatigue":** feelings of tiredness. Acidosis caused by lactic acid → brain, which produces fatigued feeling. May be a protective mechanism to prevent muscle damage.

Cardiac Muscle

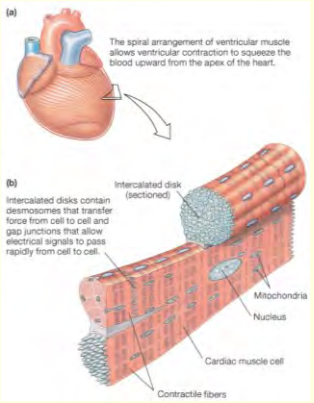
- Found only in the heart
- Develops tension at a rate intermediate to that found in skeletal and smooth muscles
- Anatomy of cardiac muscle cells
  - **a.** Similar to skeletal muscle in that it: Is striated. Has sarcomere structure
  - b. Differs from skeletal muscle in that: Muscle fibers are shorter. Fibers may be branched. Have a single nucleus.
  - **c. Similar to smooth muscle** in that: Fibers are electrically coupled by **intercalated disks.** These disks help transmit the force of contraction and, like smooth muscle, get contraction as a unit.



Adopted from Fox 2009

The Cardiac Muscle

- **1.** ~ 1% of the cardiac muscle is specialized to generate action potentials spontaneously and without nervous system input - **pacemaker cells** (autorhythmic cells)
  - *Pacemaker cells have no organized sacromeres and do not contribute to the contractile force of the heart*
- **2.** Rest of cardiac muscle contains striated myocardial cells that are connected tightly together (**desmosomes**) at structures called ***intercalated disks***. At the intercalated disks lie large number of **gap junctions** that serve to functionally connect large number of cardiac myocytes.
- **3.** Though it is striated like skeletal muscle, recall that in myocardial cells:
  - T-tubules are larger and branched
  - sarcoplasmic reticulum is smaller, creating a dependence upon extracellular $Ca^{2+}$for initiation of contraction in the heart
- **4.** Contraction of cardiac muscle occurs by the same sliding filament mechanism as in skeletal muscle. In both an action potential is needed to initiate the contraction process.
  - in skeletal muscle: action potential => Ca^{2+}release from sarcoplasmicreticulum
  - in heart muscle: action potential => opens voltage-gated Ca^{2+}channels



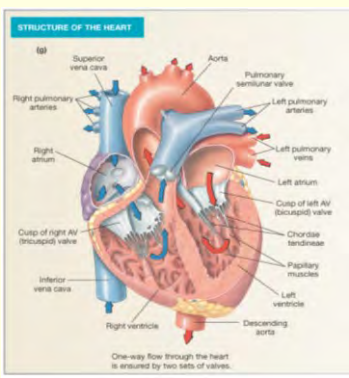
Adopted from Silverthorn 2010

The Heart

Composed primarily of:

- **Myocardium**(cardiac muscle)
- covered by thin layer of epithelium and connective tissue
  - **Endocardium (inner)**
  - **Epicardium (outer)**
- Encased in:
- **Pericardium**: tough membranous sac filled with **pericardial fluid** that lubricates heart during beating
  - **Pericarditis = inflammation of pericardium**
- Major blood vessels, the **aorta** and the **pulmonary trunk**, emerge from the top of the heart and pump blood to the tissues and lungs, respectively.
- The **coronary arteries** and **coronary veins** that supply the heart muscle with blood run across the surface of the ventricles. *Blockage in the coronary arteries is the typical cause of heart attacks.*

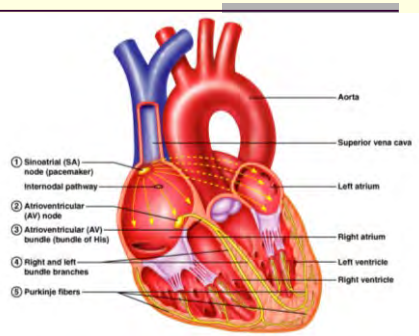
- **Valves ensure one-way flow**
  - **Atrioventricular(AV) valves**: between atria and ventricles, allow blood flow only in that direction *tricuspid valve*: three flap valve lying between right atrium & ventricle *bicuspid valve*: two flap valve between left atrium & ventricle
  - **Semilunar valves**: the *aortic valve* (between left ventricle & aorta) and the *pulmonary valve* (between right ventricle & pulmonary trunk)



Adopted from Silverthorn 2010

Conduction System

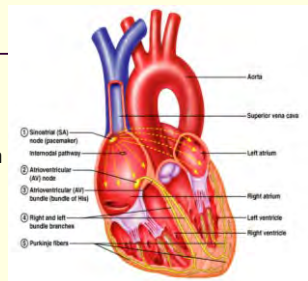
- Pacemaker cells
  - Spontaneously depolarizing membrane potentials to generate action potentials
  - Coordinate and provide rhythm to heartbeat
- Conduction fibers
  - Rapidly conduct action potentials initiated by pacemaker cells to myocardium
  - Conduction velocity = 4 m/s
  - Ordinary muscle fibers, CV = 0.4 m/s

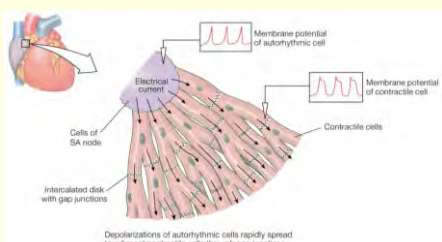


Adopted from Fox 2009

Spread of Excitation

- **Interatrial Pathway**
  - SA Node → right atrium → left atrium
  - Rapid
  - Simultaneous contraction right and left atria
- **Internodal Pathway**
  - SA Node → AV Node
  - AV Node Transmission
    - Only pathway from atria to ventricles
    - Slow conduction - AV Nodal Delay = 0.1 sec
    - Atria contract before ventricles
- **Ventricular Excitation**
  - Down Bundle of His
  - Up Purkinje Fibers
    - Purkinje Fibers contact ventricle contractile cells
    - Ventricle contracts
      - from apex up

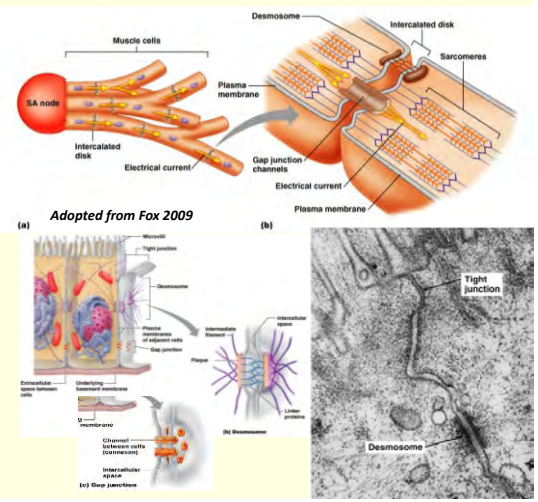




Adopted from Silverthorn 2010

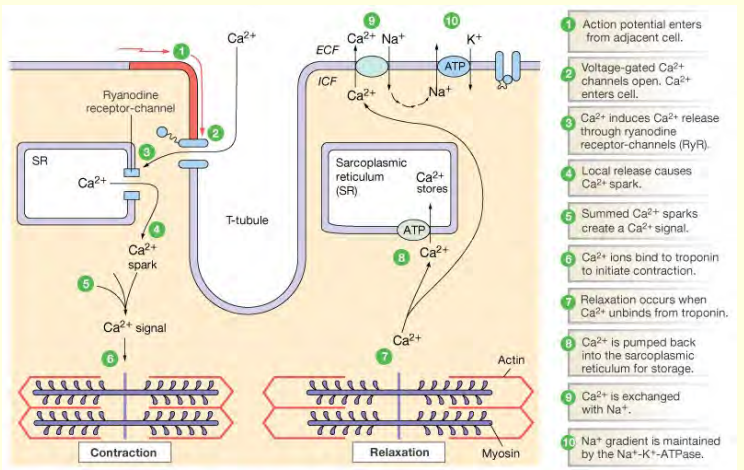
Spread of Excitation Between Cells

- Atria contract first followed by ventricles
- Coordination due to presence of gap junctions and conduction pathways
- **Intercalated disks**
  - Junctions between adjacent myocardial cells
  - Desmosomes to resist mechanical stress
  - Gap junctions for electrical coupling



Adopted from Fox 2009

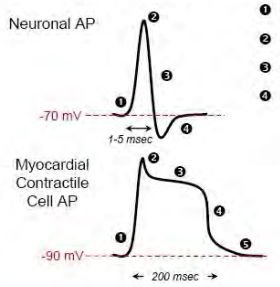
Excitation-Contraction Coupling in Cardiac Muscle



Adopted from Silverthorn 2010

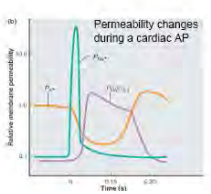
Action potentials in cardiac muscle cells

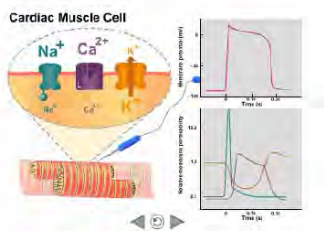
Unlike most neurons, Ca^{2+} plays an important role in the action potential in myocardial cells



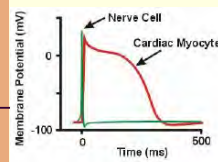
❶ Na^+ channels open
❷ Na^+ channels inactivate
❸ K^+ channels open
❹ K^+ channels close (slowly); Na/K pump active

❶ Na^+ channels open
❷ Na^+ channels inactivate; repolarizes due to open K channels
❸ Ca^{2+} channels open; fast K^+ channels close
❹ Ca^{2+} channels close; slow K^+ channels open
❺ K^+ channels close; cell returns to resting potential





9

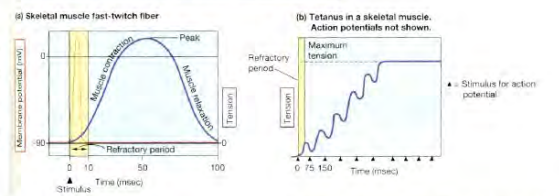


Why are longer action potentials in cardiac cells needed?

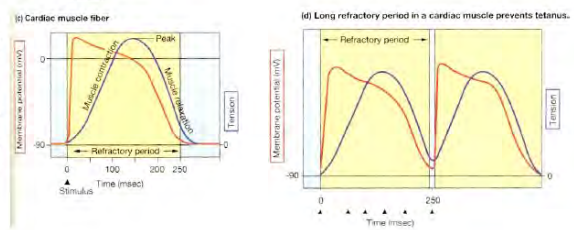
- Longer action potentials → prevent the development of **tetanus**
- Tetanus is inappropriate for the heart because the ventricles must relax between contractions so they can fill with blood.

Why do longer action potentials prevent tetanus?

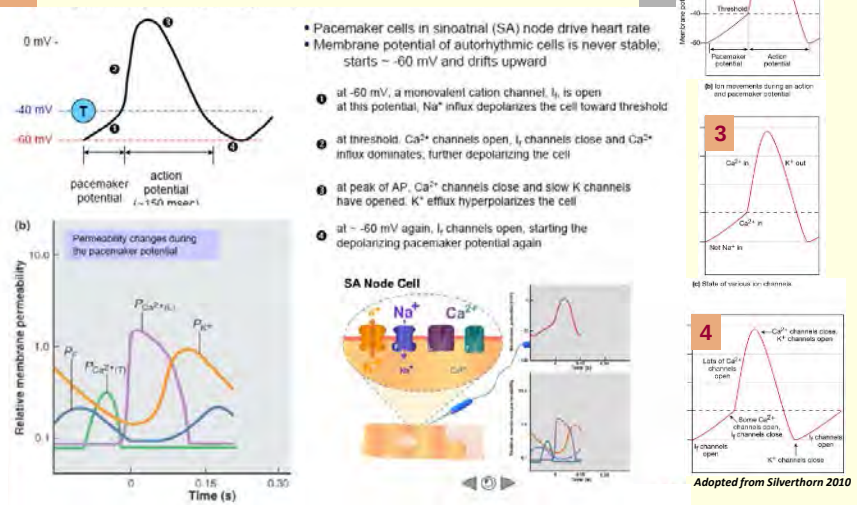
- In skeletal muscle, AP is ending as muscle contraction is beginning
- Thus, 2nd AP fired after brief refractory period will lead to summation



- In cardiac muscle, refractory period and contraction end almost simultaneously
- By the time a 2nd action potential can take place the muscle has almost completely relaxed



Action potentials in pacemaker (autorhythmic) cells

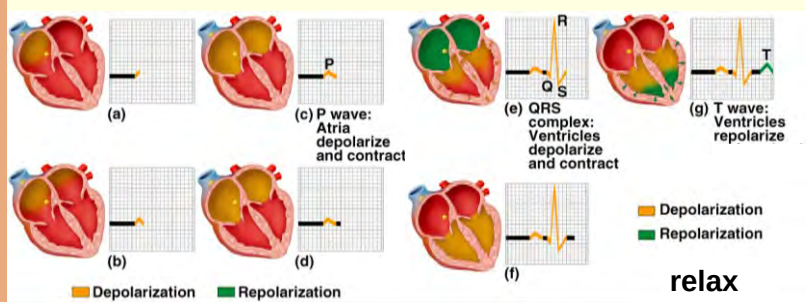


Adopted from Silverthorn 2010

Ionic Bases of the Autorhythmic Cell Action Potential

Autorhythmic cell potential change	Ion channel gating	Ion movement
Pacemaker potential		
Initial period of spontaneous depolarization to subthreshold	Funny channels open HCN channels	Sodium moves in, potassium moves out
Latter period of spontaneous depolarization to threshold	T-type calcium channels open	Calcium moves in
Rapid depolarization phase of action potential	L-type calcium channels open	Calcium moves in
Repolarization phase of action potential	Potassium channels open	Potassium moves out

The relationship between spread of excitation and ECG



Adopted from Fox 2009

Pacemaker and Heart Rate

- SA node normally sets the heart rate.
  fastest of the pacemakers (~70 beats/minute)
- AV node also has pacemaker (autorhythmic) cells
  beats more slowly (~35-40 beats/minute)
- Purkinje fibers also have autorhythmic activity

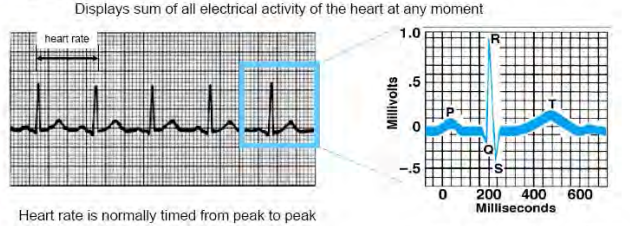
} Fastest sets the pace

"complete heart block" (AV node block)

Conduction of signals from atria to ventricles is inhibited
Atria beat at 70 beats/min; ventricles beats at 40 beats/min
too slow to maintain blood flow → insertion of artificial pacemaker

- **Electrocardiogram**: tool to measure electrical activity of the heart

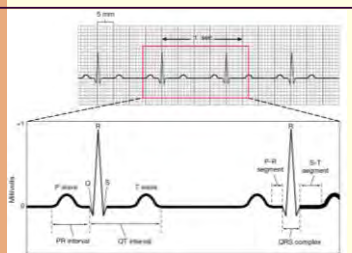
Displays sum of all electrical activity of the heart at any moment



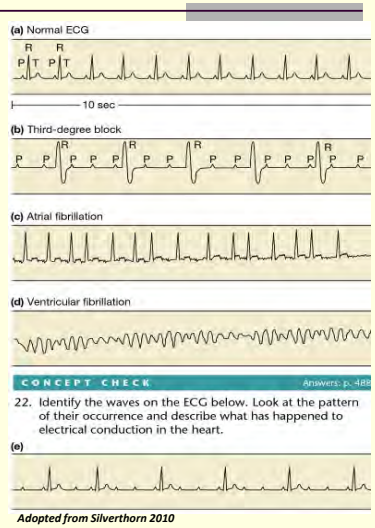
Heart rate is normally timed from peak to peak of QRS complex or beginning of P wave to beginning of next P wave

3 major components of an ECG:
P wave --- QRS complex --- T wave

ECG Information Gained



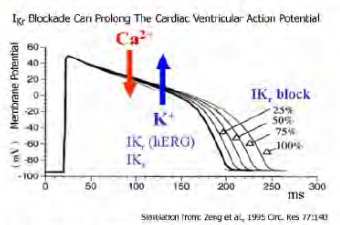
- (Non-invasive)
- Heart Rate
- Signal conduction
- Heart tissue
- Conditions



Adopted from Silverthorn 2010

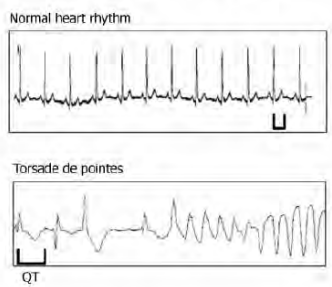
Clinical Connections

The hERG channel is an important potassium (K) channel in the cardiac cycle timing. K channels contribute to the final phase of the action potential that returns the cell to its resting state.



I_{Kr} blockade can prolong the cardiac ventricular action potential. Activation of Na and Ca currents is responsible for the depolarizing upswing (or spike) of the action potential. Activation of K channels and inactivation of these Na and Ca channels is responsible for the repolarizing decay of the action potential. Prolonging the cardiac action potential due to block of hERG channels increases the QT interval and can lead to harmful arrhythmia.

Blockade of hERG Can Cause Long QT Syndrome



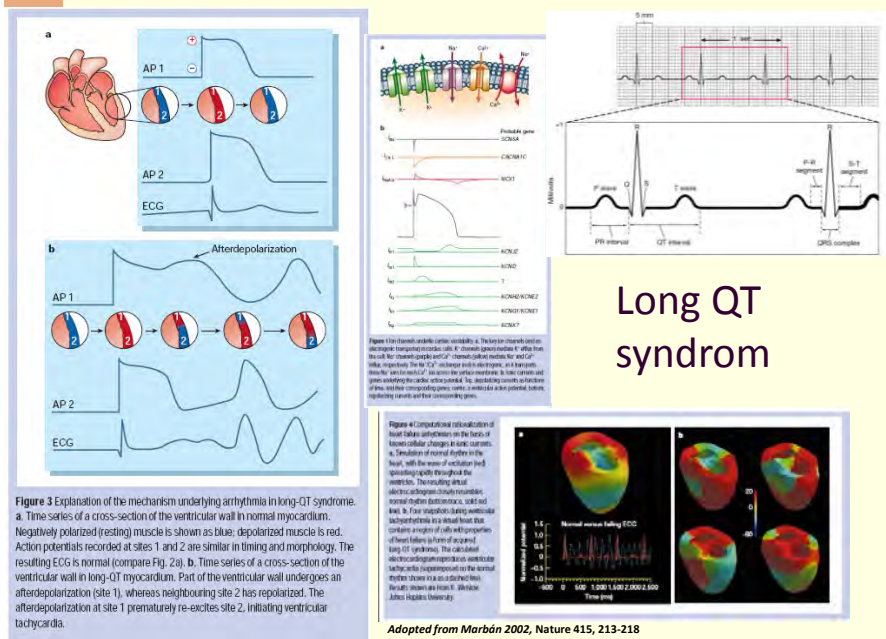


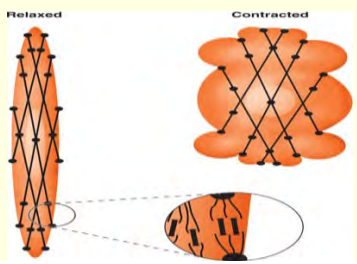
Figure 3 Explanation of the mechanism underlying arrhythmia in long-QT syndrome. **a**, Time series of a cross-section of the ventricular wall in normal myocardium. Negatively polarized (resting) muscle is shown as blue; depolarized muscle is red. Action potentials recorded at sites 1 and 2 are similar in timing and morphology. The resulting ECG is normal (compare Fig. 2a). **b**, Time series of a cross-section of the ventricular wall in long-QT myocardium. Part of the ventricular wall undergoes an afterdepolarization (site 1), whereas neighbouring site 2 has repolarized. The afterdepolarization at site 1 prematurely re-excites site 2, initiating ventricular tachycardia.

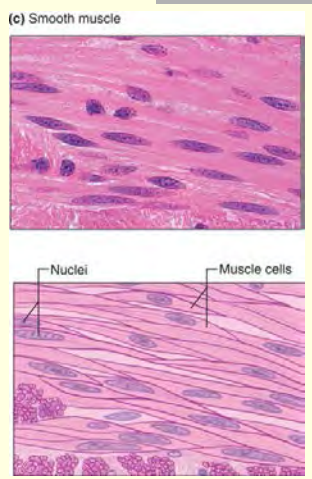
Long QT syndrom

Adopted from Marbán 2002, Nature 415, 213-218

Smooth Muscle - Anatomy

- Small, spindle shaped
- Not striated
  - actin & myosin are not organized into sarcomeres;
  - Actin & myosin are arranged in long bundles around the periphery of the cell
  - contraction causes the cell to form a globular shape rather than just shorten

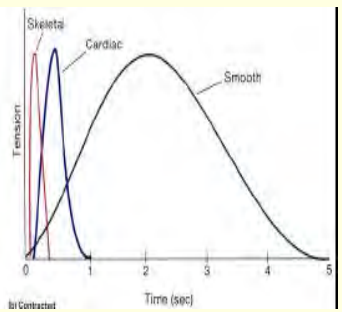




Adopted from Fox 2009

Smooth Muscle – Basic structure/function

- Found in walls of hollow organs, tubes and blood vessels
  - contraction changes the shape of th organ or tube
- Develops tension slowly compared t skeletal or cardiac muscle and relaxe more slowly
  - **a.** Can hold tension for long periods of time w/o fatigue
  - **b.** Many types are **tonically contracted**.



Types of smooth muscle

A. Single unit (visceral) smooth muscle

i. Forms walls of blood vessels, intestinal tract, ureter, respiratory tract

ii. Smooth muscle cells are electrically coupled by gap junctions.

►allows tissue to contract as a single unit

►allows tissue to display rhythmic contractions (►peristalsis)

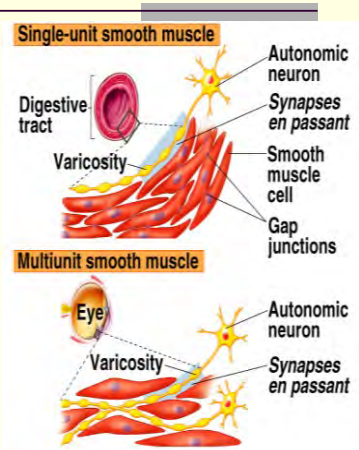
iii. Cannot recruit more fibers to vary contraction strength, controlled only by the amount of Ca^{2+}entry.

B. Multi-unit smooth muscle

i. Found in the iris and ciliary body of the eye, male reproductive tract, uterus, and large airways

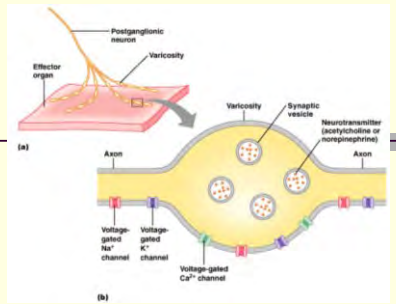
ii. Not electrically coupled; each cell must be innervated by an axon terminal

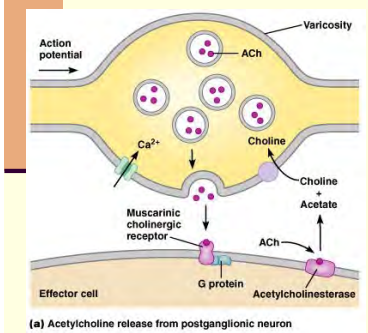
iii. Recruitment of additional fibers can increase force of contraction.



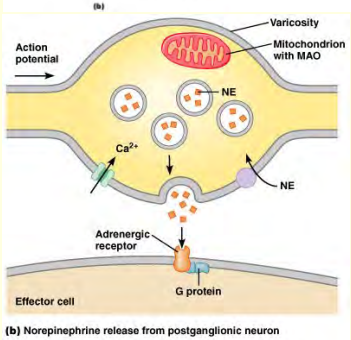
Adopted from Fox 2009

Varicosity





(a) Acetylcholine release from postganglionic neuron

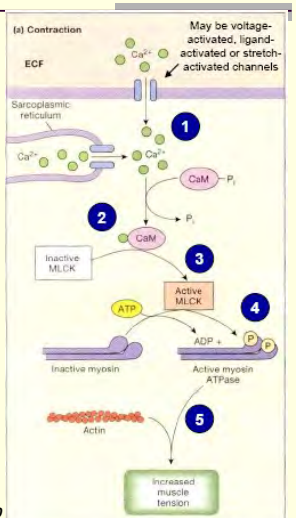


(b) Norepinephrine release from postganglionic neuron

Adopted from Fox 2009

Smooth muscle contraction

1. Signal to initiate contraction is increase in intracellular [Ca^{2+}]
   -enters from extracellular fluid and from release from SR
2. Ca^{2+}binds to **calmodulin**(CaM)
3. Ca^{2+}-calmodulin complex activates **myosin light chain kinase**(MLCK)
4. MLCK activates myosin ATPase by *phosphorylating* light proteins in myosin heads
5. When the myosin ATPase is active actin bonding and cross bridge formation can occur



Adopted from Silverthorn 2010

Comparison of the three types of muscle

CHARACTERISTIC	SKELETAL MUSCLE	Smooth Muscle: SINGLE UNIT	Smooth Muscle: MULTIUNIT	CARDIAC MUSCLE
Thick and thin filaments	Yes	Yes	Yes	Yes
Sarcomeres—banding pattern	Yes	No	No	Yes
Transverse tubules	Yes	No	No	Yes
Sarcoplasmic reticulum (SR)*	++++	+	+	++
Gap junctions between cells	No	Yes	Few	Yes
Source of activating calcium	SR	SR and extracellular	SR and extracellular	SR and extracellular
Site of calcium regulation	Troponin	Myosin	Myosin	Troponin
Speed of contraction	Fast-slow	Very slow	Very slow	Slow
Spontaneous production of action potentials by pacemakers	No	Yes	No	Yes in certain fibers, but most not spontaneously active
Tone (low levels of maintained tension in the absence of external stimuli)	No	Yes	No	No
Effect of nerve stimulation	Excitation	Excitation or inhibition	Excitation or inhibition	Excitation or inhibition
Physiological effects of hormones on excitability and contraction	No	Yes	Yes	Yes
Stretch of cell produces contraction	No	Yes	No	No

*Number of plus signs (+) indicates the relative amount of sarcoplasmic reticulum present in a given muscle type.

Adopted from Fox 2009

Electrophysiology

Patch Clamp

- Method for a cell current measurement
- We can measure currents either in whole cell or single channel configuration

Nobelova price in physiology 1991: Erwin Neher and Bert Sakmann *"for their discoveries concerning the function of single ion channels in cells"*

Erwin Neher

Bert Sakmann

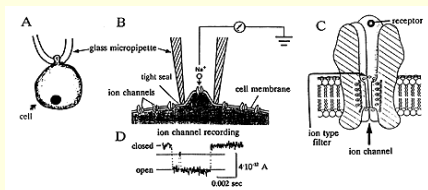
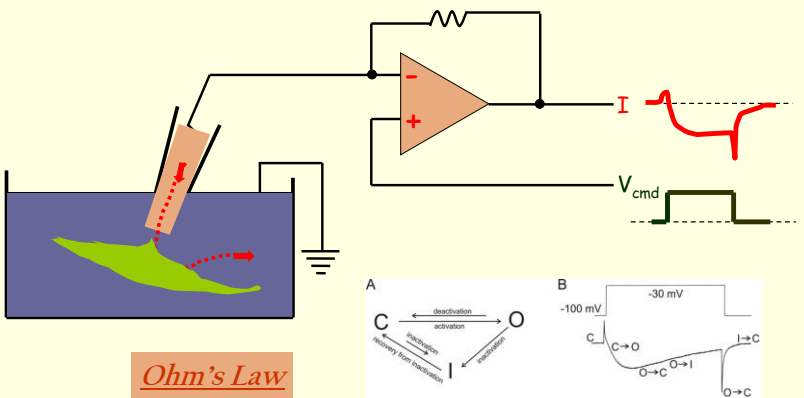


Figure 1. Registration of the flow of current through single ion channels using the recording technique of Neher and Sakmann. **A** schematically shows how a glass micropipette is brought in contact with the cell, and **B**, using a higher magnification, a part of the cell membrane, with ion channels, in close contact with the tip of the pipette. The interior of the pipette is connected to an electronic amplifier. **C** shows a channel in greater magnification with its receptor facing the exterior of the cell and its ion filter. **D** shows the current passing through the ion channel as it opens.
Adopted from Press release by the Nobel Assembly at the Karolinska Institute 1991

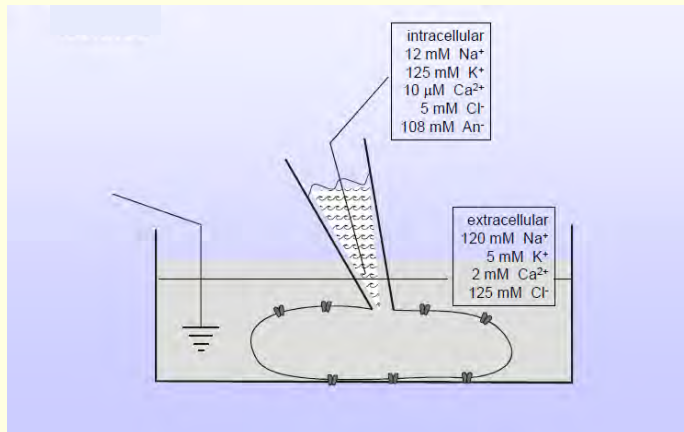
Patch clamp – whole cell configuration



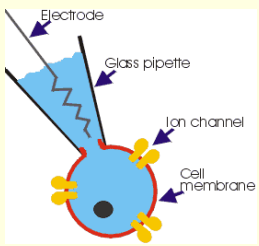
Ohm's Law

$I = V/R$

Individual ion currents must be isolated

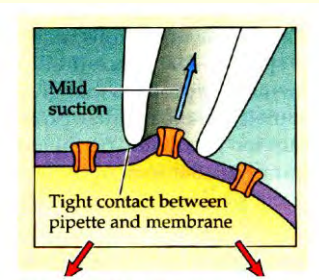


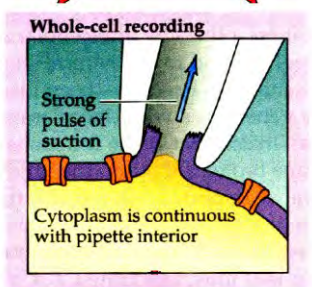
Patch Clamp



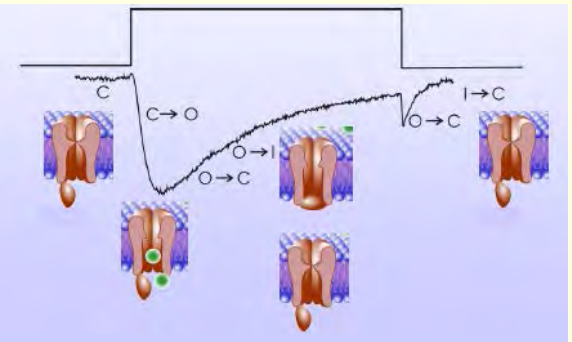
Whole Cell

- Cell-attached + suction
  - After tight seal is created, cell membrane is breached by either suction or strong electric pulse
  - Effective voltage clamp of small size cells.





Ion channels states during depolarization

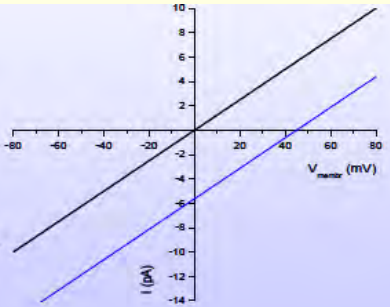


During membrane depolarization ion channels cycle between closed, open, and inactivation states

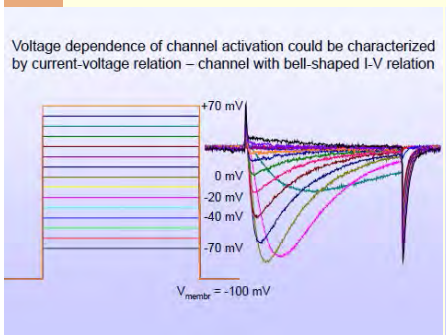
Transmembrane potential is responsible for electrical driving force

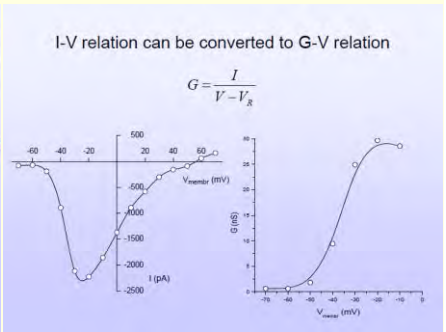
$$I = \frac{U}{R}$$

$$I = \frac{V - V_R}{R}$$

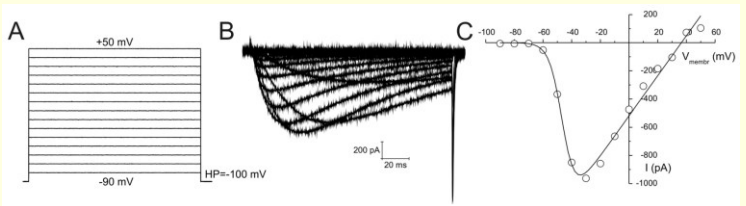


Current – voltage relationship





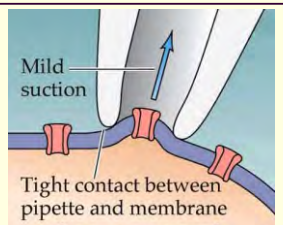
Example of whole-cell currents.

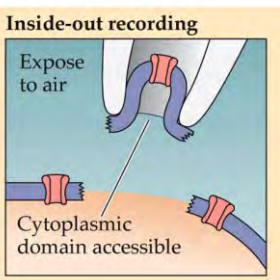


Voltage protocol used for measurement of current-voltage (I-V) relations.

Adopted from Karmažínová and Lacinová 2010.

Patch clamp measurements

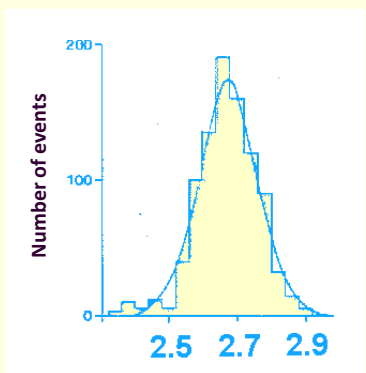


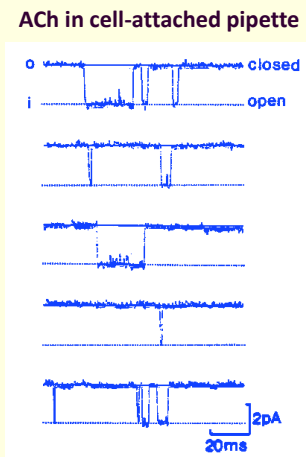


- **Cell-attached (on-cell):** pipette is in tight contact with cell membrane - **single-channel measurement**
- **Inside out**: After creating a tight seal, pipette is moved away from the cell with a patch of cell membrane at the tip. This exposes cytoplasmic side of cell membrane - **single-channel measurement**

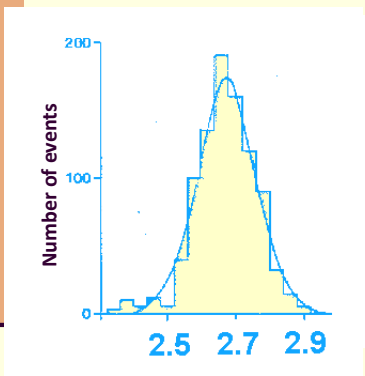
Ion channel properties

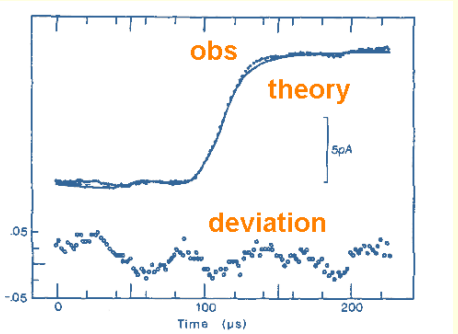
- Channels have a fixed size





Ion channel properties



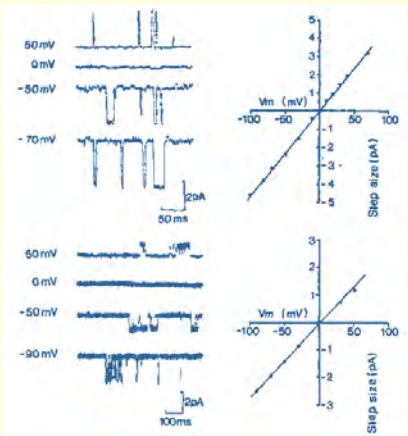


- I = 2.7pA
  ⇔ 1.6 •10^7 ions/s
  ⇔ 1.6 •10^4 ions/ms
- Rate of opening and closing is high

Ion channels & Ohm's Law

- V = IR ⇔ I = V/R
- g is a conductance
- I = V g ⇔ g = I/V
  - g is measured in Siemens

$$g = \frac{2\times10^{-9}\,\mathrm{A}}{50\times10^{-3}\,\mathrm{V}} = 40\,\mathrm{pS}$$

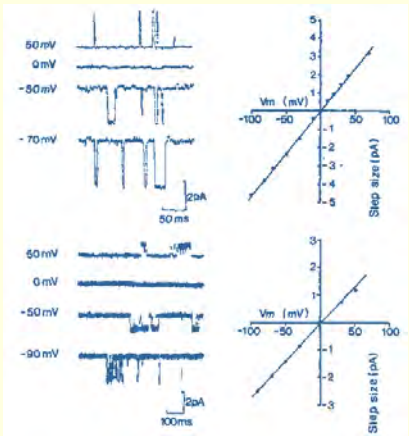


Ion channels & Ohm's Law

- High current
- Straight line of Ohm's law

It means that

- Ions do not interact with a channel pore
  - No carrier
  - No pump
  - Just a hole



Patch Clamp

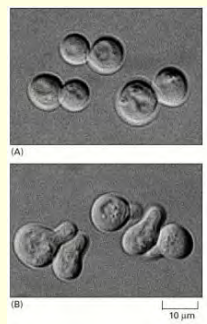
- By patch clamp approach we can assess:
  - Molecular distribution of ion channels
  - Channel opening and closing probabilities
  - Specificity towards ions
  - Modulation

Cell Signaling – Intercellular Communication

Local Signaling

General Principles of Cell Communication

- Mechanisms enabling one cell to influence the behavior of another almost certainly existed in the world of unicellular organisms long before multicellular organisms appeared on Earth.
- Evidence comes from studies of present-day unicellular eucaryotes such as yeasts.
- In the budding yeast *Saccharomyces cerevisiae*, for example, when a individual is ready to mate, it secretes a peptide *mating factor* that signals cells of the opposite mating type to stop proliferating and prepare to mate.
- Studies of yeast mutants that are unable to mate have identified many signaling molecules that are required in the signaling process. These proteins form a signaling network that includes cell-surface receptors,



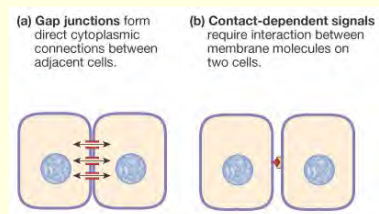
Adopted from Alberts 2008

Cell Signaling – Intercellular Communication:

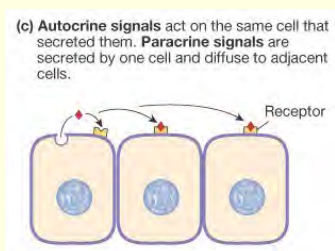
- Is how cells communicate with each other
- **Chemical**
  - Autocrine & Paracrine: local signaling
  - Endocrine system: distant signaling, diffuse target
- **Electrical**
  - Gap junction: local signaling
  - Nervous system: fast, specific, distant target

Local Signaling

- **Gap junctions**
  - Protein channels - connexin
- Direct flow to neighbor
  - Electrical- ions (charge)
  - Signal chemicals
- **Cell Adhesion Molecules (CAMs)**
  - Need direct surface contact
  - Signal chemical



- Local communication
- Signal chemicals diffuse to target
- Example: Cytokines
  - **Autocrine** –receptor on same cell
  - **Paracrine** –neighboring cells



Adopted from Fox 2009

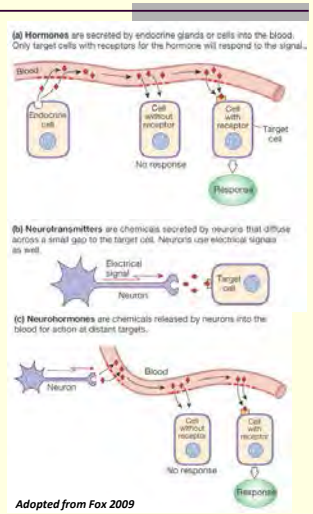
Long Distance Communication

Hormones

- Signal Chemicals
- Made in endocrine cells
- Transported via blood
- Receptors on target cells

Neurons and Neurohormones

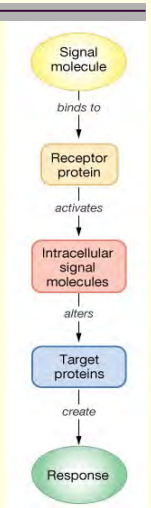
- Neurons
  - Electrical signal down axon
  - Signal molecule (**neurotransmitter**) to target cell
- **Neurohormones**
  - Chemical and electrical signals down axon
  - Hormone transported via blood to target



Adopted from Fox 2009

Cell Signal Pathways

- Signal molecule (ligand)
  - The ***extracellular signal molecule*** often act at very low concentrations (typically ≤ 10^{-8} M),
- Receptor
  - recognizes them usually bind them with high affinity (affinity constant $K_\alpha \geq 10^{-8}$ M)
- Intracellular signal
  - a cascade of intracellular signals that alter the behavior of the cell
- Target protein
- Response



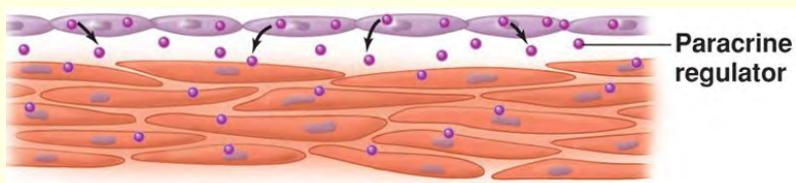
Adopted from Silverthorn 2010

Autocrine and Paracrine Regulation

- ***Autocrine regulatory molecules:***
  - Produced and act within the same tissue of an organ.
    - All autocrine regulators control gene expression in target cells.
- ***Paracrine regulatory molecules :***
  - Produced within one tissue and regulate a different tissue of the same organ.
- Cytokines (lymphokines):
  - Regulate different cells (interleukins).
- Growth factors:
  - Promote growth and cell division in any organ.
- Neutrophins:
  - Guide regenerating peripheral neurons.

Cell Signaling – Intercellular Communication:

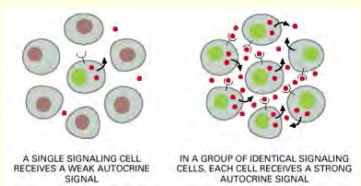
- To respond to a chemical signal, a target cell must have a receptor protein for it
- In paracrine signaling, cells secrete regulatory molecules that diffuse to nearby target cells
  - Endothelial cells produce NO to cotrol a muscle tone in vascular smooth muscle cells



Adopted from Fox 2009

Autocrine Signaling Can Coordinate Decisions by Groups of Identical Cells

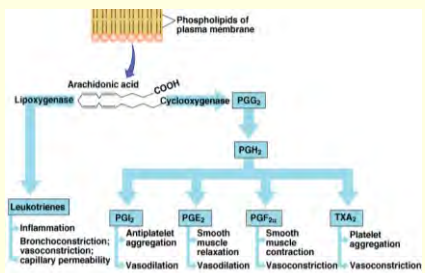
- ***Autocrine signaling*** - a cell secretes signaling molecule that can bind back to its own receptors.
  - During development, for example, once a cell has been directed along a particular pathway of differentiation, it may begin to secrete autocrine signals to itself that reinforce this developmental decision.
- A group of identical cells produces a higher concentration of a secreted signal than does a single cell. When this signal binds back to a receptor on the same cell type, it encourages the cells to respond coordinately as a group.
- ***Autocrine signaling*** is most effective when performed simultaneously by neighboring cells of the same type, and it is likely to be used to encourage groups of identical cells to make the same developmental decisions.
  - Thus, it is thought to be one possible mechanism underlying the "community effect" that is observed in early development, during which a group of identical cells can respond to a differentiation -inducing signal but a single isolated cell of the same type cannot .
  - Unfortunately, cancer cells often use autocrine signaling to overcome the normal controls on cell proliferation and survival.



Adopted from Alberts 2008

Prostaglandins

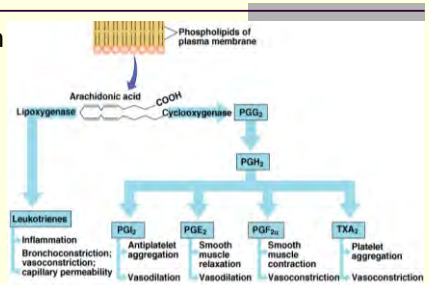
- Most diverse group of **autocrine regulators**.
- Produced in almost every organ and have wide variety of functions.
- Different prostaglandins may exert antagonistic effects in some tissues.
  - Immune system:
    - Promote inflammatory process.
  - Reproductive system:
    - Play role in ovulation.
  - Respiratory system:
    - May bronchoconstrict or bronchodilate.
  - Circulatory system:
    - Vasoconstrictors or vasodilators.
  - Urinary system:
    - Vasodilation.
  - Digestive system:
    - Inhibit gastric secretion.



Adopted from Fox 2009

Prostaglandins

- Inhibitors of prostaglandin synthesis:
  - Non-steroidal anti-inflammatory drugs (NSAIDS).
    - Aspirin, indomethacin, ibuprofen: inhibit COX1.
  - Celecoxib and rofecoxib: inhibit COX2.



Preventing platelet aggregation in healthy blood vessel

Healthy Endothelial Cells

Arachidonic Acid

↓

Prostacyclin (Prostaglandin I_2)

Inhibits Platelet Aggregation

Adhered Platelets

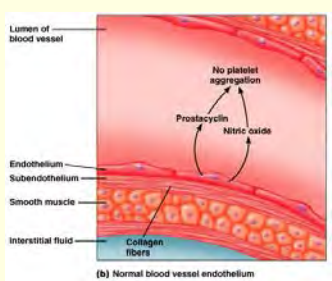
Arachidonic Acid

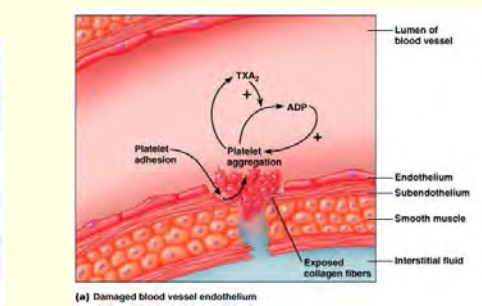
↓

Thromboxane A_2

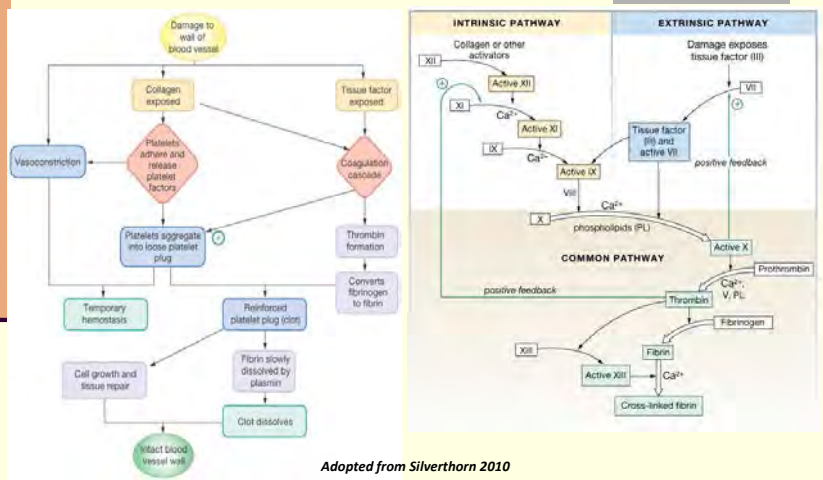
Stimulates Platelet Aggregation and Secretions

Adopted from Fox 2009





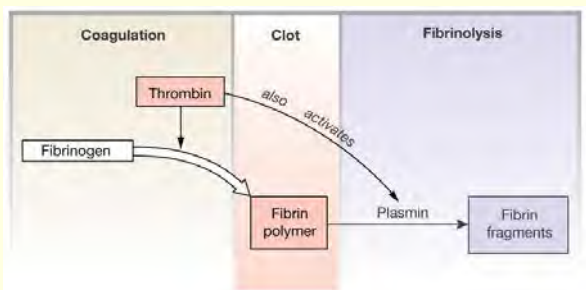
Overview of Hemostasis: Clot Formation & Vessel Repair



Adopted from Silverthorn 2010

Dissolution of Clots

- When damage is repaired, activated factor XII causes activation of kallikrein
  - Kallikrein converts plasminogen to plasmin
    - Plasmin digests fibrin, dissolving clot

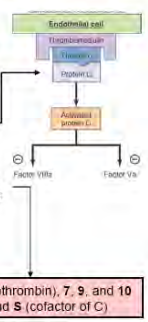


Inhibition of clot formation

The extent of blood clotting is limited by:

1. Inhibition of platelet adhesion
   example: ***prostacyclin*** in blood vessel epithelium limits clot site to area of tissue damage

2. Inhibition of the coagulation cascade and fibrin production
   Anticoagulants: released by endothelial cells
   - most block one of the reaction steps in the coagulation cascade
   - endogenous anticoagulants:
     Heparin, Antithrombin III } Block active factors IX, X, XI, XII
     Protein C: inhibits clotting factors V (directly) & VIII (indirectly)
   - drugs:
     Coumarin, *Wayfarin* } Block the action of the cofactor, vitamin K, that is needed in the formation of several clotting factors
     Aspirin (salicylic acid): prevents platelet plug formation



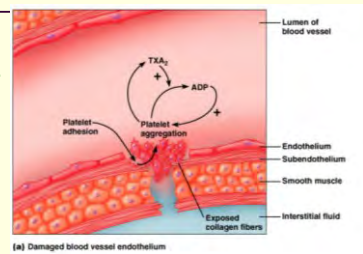
also: a rat poison → uncontrollable bleeding when eaten...

factors **2** (prothrombin), **7**, **9**, and **10** proteins **C** and **S** (cofactor of C)

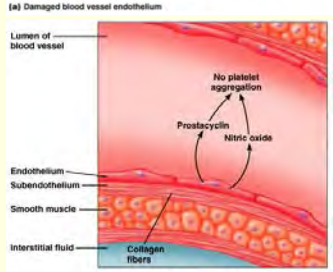
Direct Thrombin Inhibitors (DTIs). Direct thrombin inhibitors are a more recent group of anti-coagulants. The first DTI, hirudin, is a natural substance derived from the saliva of leeches.

Aspirin as an Anticoagulant

- Low doses – anticoagulant
  - Inhibits formation of thromboxane A_2
- High doses – coagulant
  - Increase likelihood of clot formation
  - Inhibits formation of prostacyclin



(a) Damaged blood vessel endothelium

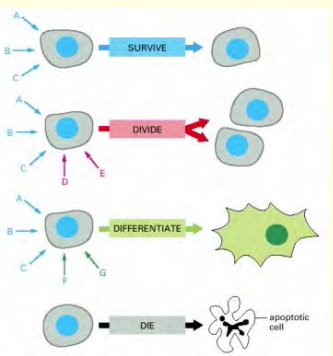


(b) Normal blood vessel endothelium

Adopted from Fox 2009

Each Cell Is Programmed to Respond to Specific Combinations of Extracellular Signal Molecules

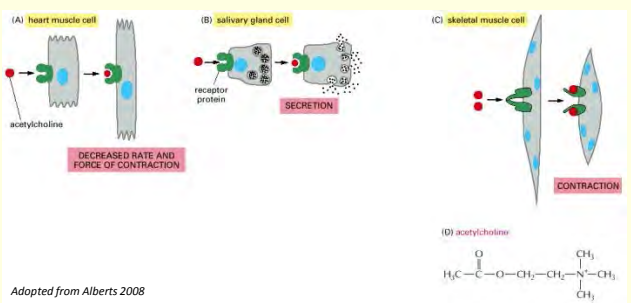
- A typical cell in a multicellular organism is exposed to hundreds of different signals in its environment.
  - They can act in many millions of combinations.
- The cell must respond to signals selectively, according to its own specific character, which it has acquired through progressive cell specialization. A cell may be programmed to respond to one combination of signals by differentiating, to another combination by multiplying, and to yet another by performing some specialized function such as contraction or secretion.
- Each cell type displays a set of receptors that enables it to respond to a corresponding set of signals produced by other cells. These *signals* work in combinations to regulate the behavior of the cell.
  - to survive
  - to divide
  - *To die.*
- An almost unlimited number of signaling combinations
  - The use of these combinations to control cell behavior enables an animal to control its cells in highly specific ways by using a limited diversity of signals.



Adopted from Alberts 2008

Different Cells Can Respond Differently to the Same Extracellular Signal Molecule

- Thus, a single ***signal*** often has different effects on different target cells.
  - ***Acetylcholine***
    - stimulates the contraction of skeletal muscle cells,
    - decreases the rate and force of contraction in heart muscle cells.
      - But receptor differences are not always the explanation for the different effects. In many cases, the same signal binds to identical receptors yet produces very different responses in different types of target cells, reflecting ***differences in the internal machinery*** to which the receptors are coupled.



Adopted from Alberts 2008

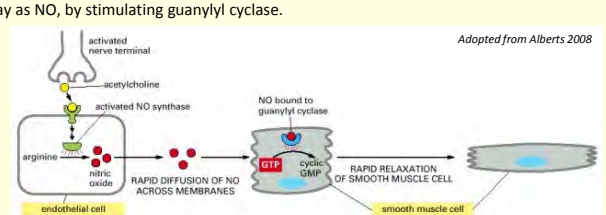
The Concentration of a Molecule Can Be Adjusted Quickly Only If the Lifetime of the Molecule Is Short

- It is natural to think of signaling systems in terms of the changes produced when a signal is delivered. But it is just as important to consider what happens when a signal is withdrawn.
  - In most cases in adult tissues, however, the response fades when a signal ceases. The effect is transitory because the signal exerts its effects by altering a set of molecules that are unstable, undergoing continual turnover..
- It is also true, although much less obvious, that this turnover rate also determines the promptness of the response when a signal is turned on. In fact, after a molecule's synthesis rate has been either increased or decreased abruptly, the time required for the molecule to shift halfway from its old to its new equilibrium concentration is equal to its normal half-life—that is, equal to the time that would be required for its concentration to fall by half if all synthesis were stopped.
  - The same principles apply to proteins and small molecules, and to molecules in the extracellular space and inside cells. Many intracellular proteins have short half-lives, some surviving for less than 10 minutes. In most cases, these are proteins with key regulatory roles, whose concentrations are rapidly regulated in the cell by changes in their rates of synthesis. Likewise, any covalent modifications of proteins that occur as part of a rapid signaling process—most commonly by phosphorylation(the addition of a phosphate group to an amino acid side chain) — must be continuously removed at a rapid rate to make rapid signaling possible.

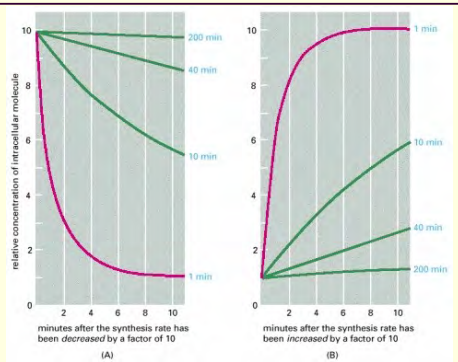
Nitric Oxide Gas Signals by Binding Directly to an Enzyme Inside the Target Cell

- Some ***signals*** are hydrophobic enough and/or small enough to pass readily across the target-cell membrane. Once inside, they directly regulate the activity of a specific intracellular protein. An important and remarkable example is the gas ***nitric oxide (NO),*** which acts as a signal in both animals and plants.
- In mammals, one of its functions is to regulate smooth muscle contraction.
  - Upon release of **NO** smooth muscle cells relax. This effect of NO on blood vessels provides an explanation for the mechanism of action of nitroglycerine.
  - Many types of neurons use NO gas to signal to their neighbors. The ***NO*** released by autonomic nerves in the penis, for example, causes the local blood vessel dilation that is responsible for penile erection. **NO** is also produced as a local mediator by activated macrophages and neutrophils to help them to kill invading microorganisms.
- In plants, **NO** is involved in the defensive responses to injury or infection.
- ***Carbon monoxide (CO)*** - is another gas that is used as an intercellular signal. It can act in the same way as NO, by stimulating guanylyl cyclase.

Adopted from Alberts 2008



The importance of rapid turnover

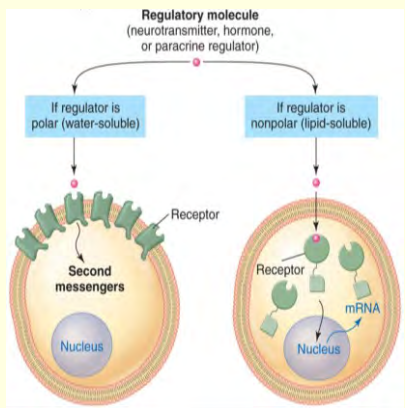


Adopted from Alberts 2008

The predicted relative rates of change in the intracellular concentrations of molecules with differing turnover times when their synthesis rates are either (**A**) decreased or (**B**) increased suddenly by a factor of 10. In both cases, the concentrations of those that are normally being rapidly degraded in the cell *(red lines)* change quickly, whereas the concentrations of those that are normally being slowly degraded *(green lines)* change proportionally more slowly. The numbers (in *blue)* on the right are the half-lives assumed for each of the different molecules.

How Regulatory Molecules Influence Target Cells

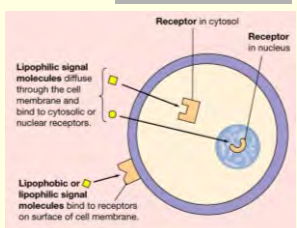
- *Nonpolar regulatory molecules* pass through plasma membrane, bind to receptors in nucleus, and affect transcription
  - Examples include steroid and thyroid hormones and nitric oxide
- *Polar regulatory molecules* bind to cell surface receptors
  - Activated receptors send 2nd messengers into cytoplasm to mediate actions of regulatory molecule
  - May be ions (e.g. Ca^{2+}) or other molecules such as cyclic AMP (cAMP) or G-proteins

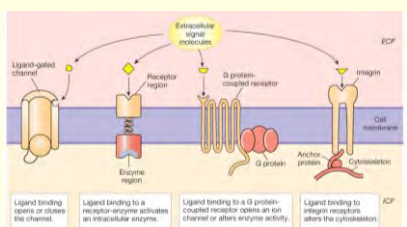


Adopted from Fox 2009

Receptor locations

- **Cytosolic or Nuclear**
  - Lipophilic ligand enters cell
  - Often activates gene
  - Slower response
- **Cell membrane**
  - Lipophobic ligand can't enter cell
  - Outer surface receptor
  - Fast response
- **Membrane Receptor Classes**
  - Ligand- gated channel
  - Receptor enzymes
  - G-protein-coupled
  - Integrin

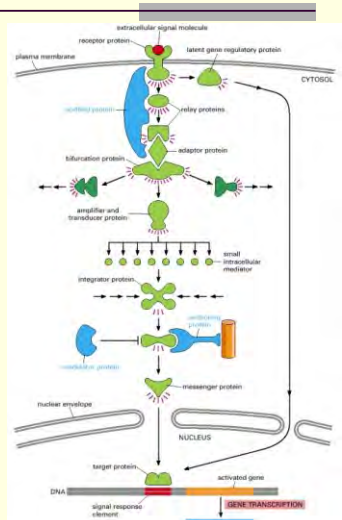




Adopted from Fox 2009

Most Activated Cell-Surface Receptors Relay Signals Via Small Molecules and a Network of Intracellular Signaling Proteins

- The *small intracellular signaling molecules* are called ***second messengers*** (the "first messengers" being the extracellular signals).
  - They are generated in large numbers in response to receptor activation and rapidly diffuse away from their source, broadcasting the signal to other parts of the cell. Some, such as *cyclic AMP* and Ca^{2+}, are water-soluble and diffuse in the cytosol, while others, such as *diacylglycerol,* are lipid-soluble and diffuse in the plane of the plasma membrane. In either case, they pass the signal on by binding to and altering the behavior of selected signaling proteins or target proteins.
- The *large intracellular signaling molecules* are intracellular signaling proteins. Many of these relay the signal into the cell by either activating the next signaling protein in the chain or generating small intracellular mediators. These proteins can be classified according to their particular function, although many fall into more than one category

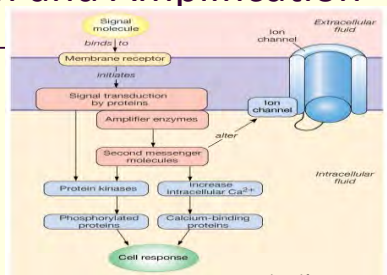


Adopted from Alberts 2008

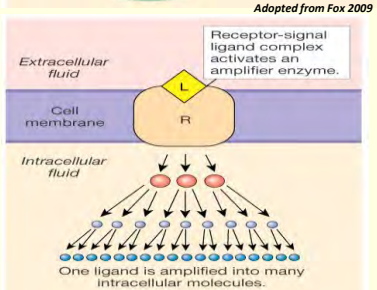
Signal Transduction and Amplification

- **Transduction**
- Transforms signal energy
- Protein kinase
- Second messenger
- Activate proteins
  - Phosporylation
  - Bind calcium
- Cell response

- **Amplification**
- Small signal produces large cell response
- Amplification enzyme
- Cascade

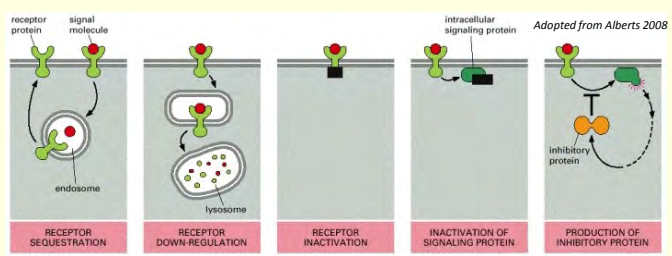


Adopted from Fox 2009



Cells Can Adjust Their Sensitivity to a Signal

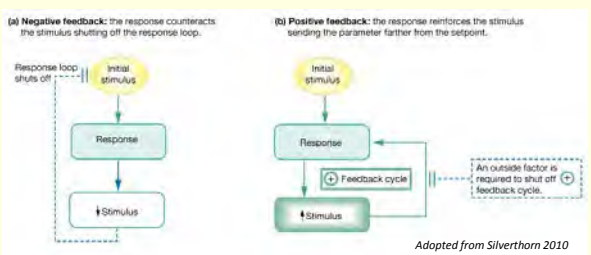
- In responding to many types of stimuli, cells and organisms are able to detect the same percentage of change in a signal over a very wide range of stimulus intensities. This requires that the target cells undergo a reversible process of ***adaptation***, or ***desentization***, whereby a prolonged exposure to a stimulus decreases the cells' response to that level of exposure. In chemical signaling, ***adaptation*** enables cells to respond to *changes* in the concentration of a signaling ligand (rather than to the absolute concentration of the ligand) over a very wide range of ligand concentrations. The general principle is one of a negative feedback that operates with a delay. A strong response modifies the machinery for making that response, such that the machinery resets itself to an off position. Owing to the delay, however, a sudden change in the stimulus is able to make itself felt strongly for a short period before
- ***Desentization*** to a signal can occur in various ways:



Adopted from Alberts 2008

Feedback Loops

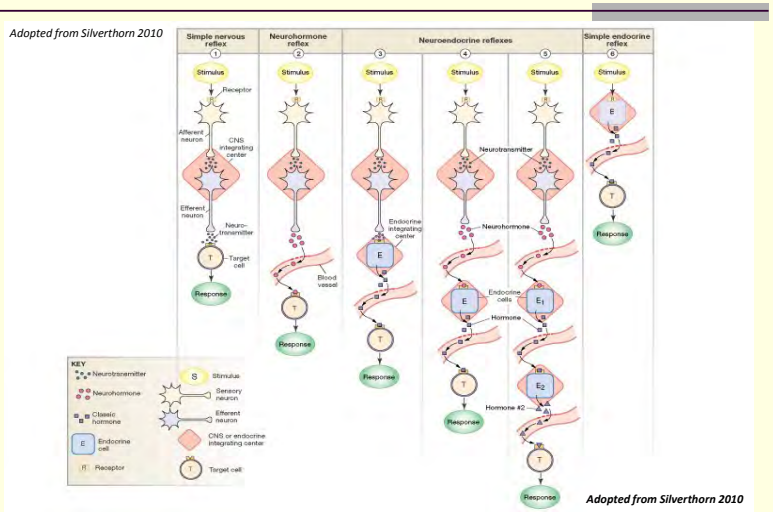
- **Negative**: are homeostatic
  - Response slows stimulation
  - Return to optimal range
- **Positive**: stimulation drives more stimulation
- **Feed forward**: prepares body for change



Adopted from Silverthorn 2010

Review of Control Pathways

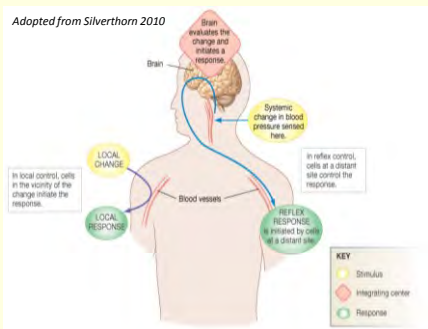
Adopted from Silverthorn 2010



Adopted from Silverthorn 2010

Homeostasis Control Pathways

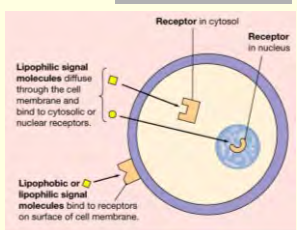
- Maintain homeostasis
- Local–paracrines
- Long-distance–reflex control
  - Nervous
  - Endocrine
  - Cytokines



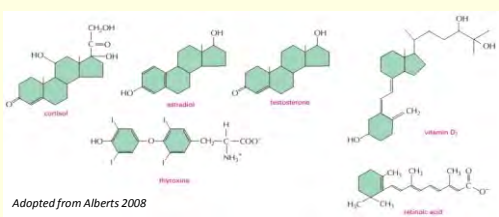
Cytosolic or Nuclear Receptors

Cytosolic or Nuclear Receptors

- **Cytosolic or Nuclear**
  - Lipophilic ligand enters cell
    - These signals include *steroid hormones*, *thyroid hormones*, *retinoids*, and *vitamin D*. Although they differ greatly from one another in both chemical structure and function, they all act by a similar mechanism.
  - Often activates gene
  - Slower response



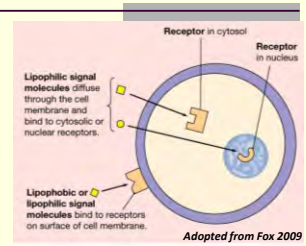
Adopted from Fox 2009



Adopted from Alberts 2008

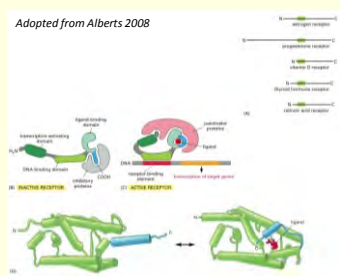
Cytosolic or Nuclear Receptors

- The intracellular receptors for the steroid and thyroid hormones, retinoids, and vitamin D all bind to specific DNA sequences adjacent to the genes the ligand regulates.
- Some receptors, such as those for cortisol, are located primarily ***in the cytosol*** and enter the nucleus after ligand binding;
- Others, such as the thyroid and retinoid receptors, are ***bound to DNA in the nucleus*** even in the absence of ligand.
  - In either case, the inactive receptors are bound to inhibitory protein complexes, and ligand binding alters the conformation of the receptor protein, causing the inhibitory complex to dissociate.
  - ***The transcriptional response*** usually takes place in successive steps:



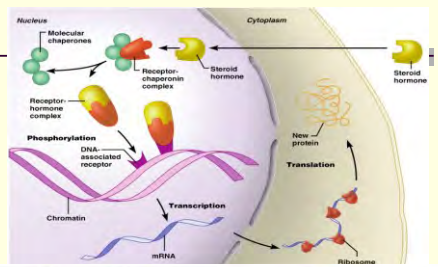
Adopted from Fox 2009

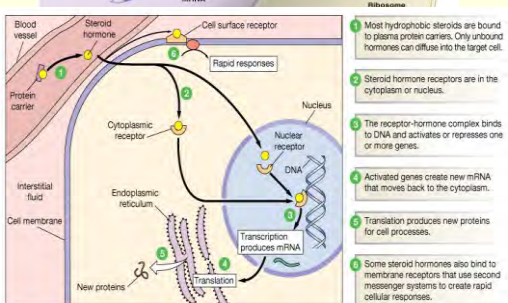
Adopted from Alberts 2008



Steroid Hormones

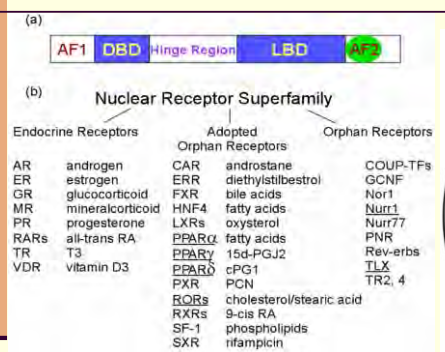
- Steroid hormones and thyroid hormone diffuse easily into their target cells
  - Genomic effects
  - Non-genomic effects
- Once inside, they bind and activate a specific intracellular receptor
- The hormone-receptor complex travels to the nucleus and binds a DNA-associated receptor protein
- This interaction prompts DNA transcription to produce mRNA
- The mRNA is translated into proteins, which bring about a cellular effect



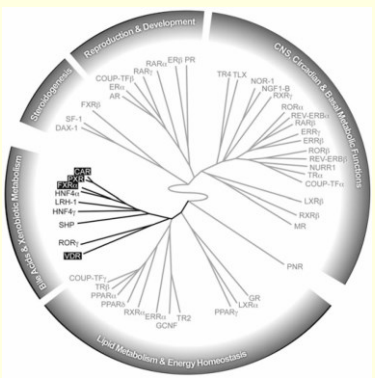


Adopted from Silverthorn 2010

Nuclear Receptor Superfamily



Structure/function domains and the superfamily members of nuclear receptors. (a) Nuclear receptor domain structure. In general, the receptor structure is comprised of an amino-terminal activation domain AF-1, DNA binding domain (DBD), a hinge region, a conserved ligand binding domain (LBD), and a variable C-terminal region with a second activation domain AF-2. **(b)** The nuclear receptor superfamily includes the endocrine receptors, the adopted orphan receptors, and the orphan receptors. *Figure adapted from Shi Y. Drug Discov Today. 2007 June ; 12(11-12): 440–445. doi:10.1016/j.drudis.2007.04.006.*

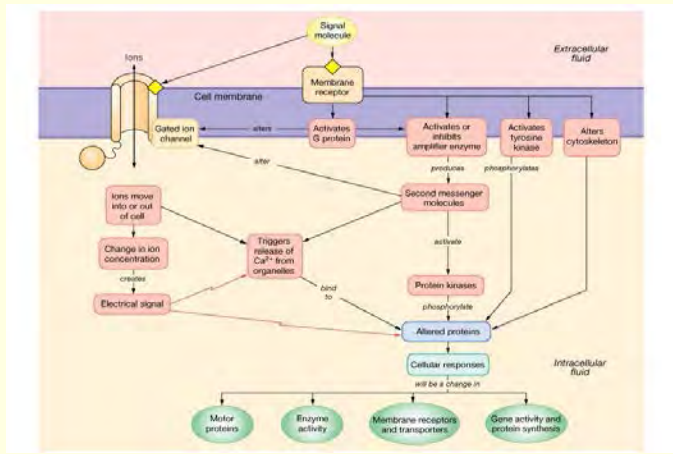


Dendrogram of the nuclear receptor superfamily.

Figure adapted from Schmidt and Mangelsdorf : Nutr Rev. 2008 October ; 66(10 Suppl 2): S88–S97.

Cell Signaling – Intracellular Communication

Transduction Reviewed

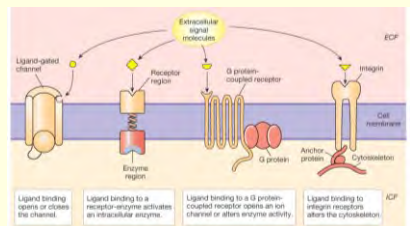


Adopted from Fox 2009

Membrane Receptors

Receptor locations

- **Cell membrane**
  - Lipophobic ligand can't enter cell
  - Outer surface receptor
  - Fast response
- **Membrane Receptor Classes**
  - Ligand- gated channel
  - Receptor enzymes
  - G-protein-coupled
  - Integrin



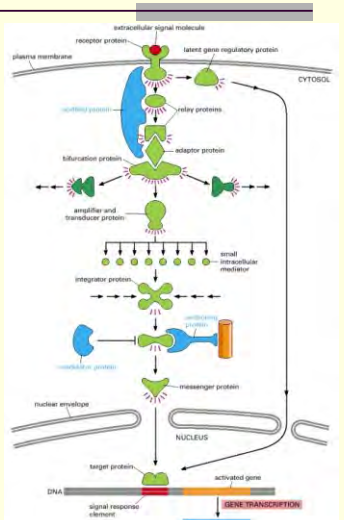
Adopted from Fox 2009

As mentioned previously, all water-soluble signal bind to specific receptor proteins on the surface of the target cells that they influence. These cell-surface receptor proteins act as ***signal transducers***. They convert an extracellular ligand-binding event into intracellular signals that alter the behavior of the target cell.

Most Activated Cell-Surface Receptors Relay Signals Via Small Molecules and a Network of Intracellular Signaling Proteins

- The *small intracellular signaling molecules* are called ***second messengers*** (the "first messengers" being the extracellular signals).
  - They are generated in large numbers in response to receptor activation and rapidly diffuse away from their source, broadcasting the signal to other parts of the cell. Some, such as *cyclic AMP* and Ca^{2+}, are water-soluble and diffuse in the cytosol, while others, such as *diacylglycerol,* are lipid-soluble and diffuse in the plane of the plasma membrane. In either case, they pass the signal on by binding to and altering the behavior of selected signaling proteins or target proteins.

- The *large intracellular signaling molecules* are intracellular signaling proteins. Many of these relay the signal into the cell by either activating the next signaling protein (G-protein cascade) in the chain or generating small intracellular mediators. These proteins can be classified according to their particular function, although many fall into more than one category.

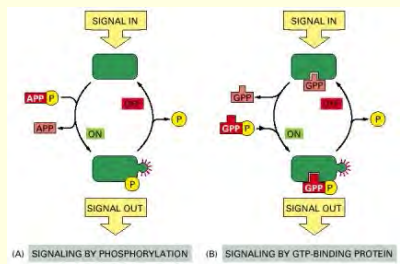


Adopted from Alberts 2008

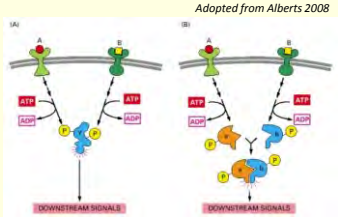
Some Intracellular Signaling Proteins Act as Molecular Switches

- Many *intracellular signaling proteins* behave like **molecular switches:** on receipt of a signal they switch from an inactive to an active state, until another process switches them off. As we discussed earlier, the switching off is just as important as the switching on. If a signaling pathway is to recover after transmitting a signal so that it can be ready to transmit another, every activated molecule in the pathway must be returned to its original inactivated state.

- Complex cell behavior, such as cell survival and cell proliferation, are generally stimulated by specific combinations of extracellular signals rather than by a single signal acting alone. The cell therefore has to integrate the information coming from separate signals so as to make an appropriate response—to live or die, to divide or not, and so on. This integration usually depends on *integrator proteins*, which are equivalent to the microprocessors in a computer: they require multiple signal inputs to produce an output that causes the desired biological effect.

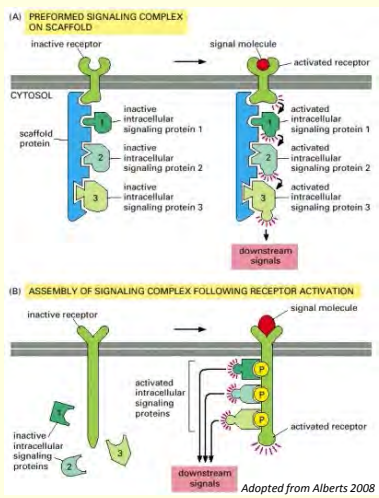


Adopted from Alberts 2008



Two types of intracellular signaling complexes

- **(A)** A receptor and some of the intracellular signaling proteins it activates in sequence are preassembled into a signaling complex by a large scaffold protein.
- **(B)** A large signaling complex is assembled after a receptor has been activated by the binding of an extracellular signal molecule; here the activated receptor phosphorylates itself at multiple sites, which then act as docking sites for intracellular signaling proteins.

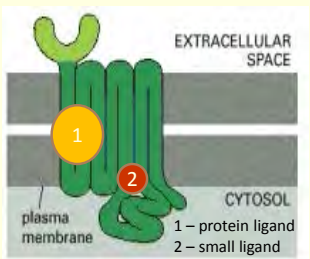


Adopted from Alberts 2008

G-protein Coupled Receptors

Signaling through G-protein linked receptors

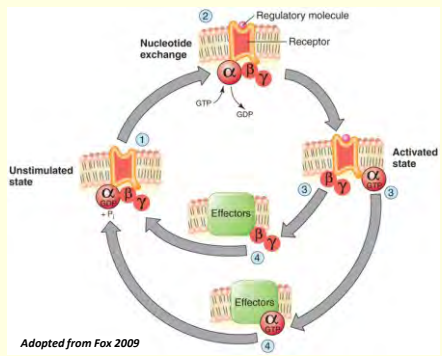
- These are by far the largest class of cell-surface receptors found in all eukaryotes, and they mediate the responses to the great majority of extracellular signals. This superfamily of receptor proteins not only mediates intercellular communication; it is also central to vision, smell, and taste perception.
- The same ligand can activate many different receptor family members; at least 9 distinct ***G-protein linked receptors*** are activated by adrenaline, for example, another 5 or more by Ach, and at least 15 by the serotonin
- Despite the chemical and functional diversity of the signal molecules that bind to them, all ***G-protein linked receptors*** have a similar structure. They consist of a single polypeptide chain that threads back and forth across the lipid bilayer seven times and are therefore sometimes called ***serpentine receptors***.
- It is remarkable that about half of all known drugs work through ***G-protein linked*** receptors. Genome sequencing projects are revealing vast numbers of new family members, many of which are likely targets for new drugs that remain to be discovered.



Adopted from Alberts 2008

G-proteins

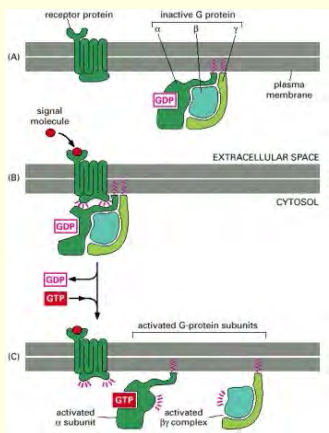
- Are part of 2nd messenger pathway in many cells
- Contain 3 subunits whose components dissociate when a cell surface receptor is activated
  - A subunit binds to an ion channel or enzyme, changing their activity



Adopted from Fox 2009

Trimeric G Proteins Disassemble to Relay Signals from G-Protein-linked Receptors

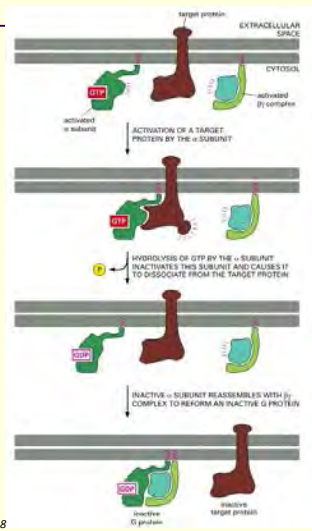
- (A) In the unstimulated state, the receptor and the G protein are both inactive. Although they are shown here as separate entities in the plasma membrane, in some cases, at least, they are associated in a preformed complex.
- (B) Binding of an extracellular signal to the receptor changes the conformation of the receptor, which in turn alters the conformation of the G protein that is bound to the receptor .
- (C) The alteration of the α γ subunits of the G protein allows it to exchange its GDP for GTP. This causes the G protein to break up into two active components—an α subunit and a βγ complex, both of which can regulate the activity of target proteins in the plasma membrane. The receptor stays active while the external signal molecule is bound to it, and it can therefore catalyze the activation of many molecules of G protein.



Adopted from Alberts 2008

Shutting off GTPase activity

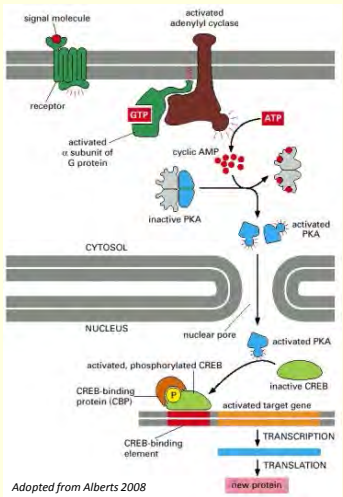
- The α subunit is a **GTPase**, and once it hydrolyzes its bound GTP to GDP, it reassociates with a βγ complex to re-form an inactive G protein, reversing the activation process.
  - The time during which the α subunit and βγ complex remain apart and active is usually short, and it depends on how quickly the α subunit hydrolyzes its bound GTP.
- An isolated α subunit is an inefficient GTPase,
  - Its activation is usually reversed much faster than this, however, because the GTPase activity of the α subunit is greatly enhanced by the binding of a second protein, which can be either its target protein or a specific modulator known as a ***regulator of G protein signaling (RGS).***
- **RGS** proteins act as α- subunit -specific GTPase *activating* proteins *(GAPs),* and they are thought to have a crucial role in shutting off *G* protein -mediated responses in all eukaryotes. There are about 25 RGS proteins encoded in the human genome, each of which is thought to interact with a particular set of *G* proteins.



Adopted from Alberts 2008

Cyclic-AMP-dependent Protein Kinase (***PKA***) Mediates Most of the Effects of Cyclic AMP (***cAMP***)

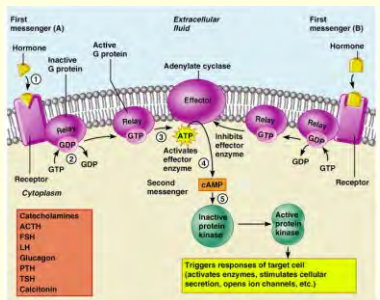
- Although cyclic AMP can directly activate certain types of ion channels in the plasma membrane of some highly specialized cells, in most animal cells it exerts its effects mainly by activating ***cyclic-AMP-dependent protein kinase (PKA)***.
  - catalyzes the transfer of the terminal phosphate group from ATP to specific serines or threonines of selected target proteins, thereby regulating their activity.
  - is found in all animal cells
    - The substrates for PKA differ in different cell types, which explains why the effects of cyclic AMP vary so markedly depending on the cell type
- Responses mediated by ***cAMP*** are rapid or slow.
  - In skeletal muscle cells - activated PKA phosphorylates enzymes involved in glycogen metabolism
    - increasing the amount of glucose available to the muscle cell within seconds
  - At the other extreme are responses that take hours to develop fully and involve changes in the transcription of specific genes.
    - In cells that secrete the peptide *somatostatin*, cyclic AMP activates the gene that encodes this hormone.



Adopted from Alberts 2008

Some G Proteins Signal By Regulating the Production of cAMP

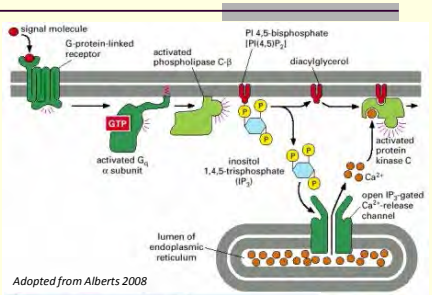
- Signal molecule (first messenger) binds to its receptor, which then binds to a G protein
  - The G protein is then activated as it binds GTP, displacing GDP
  - Activated G protein activates the effector enzyme adenylate cyclase
  - Adenylate cyclase generates cAMP (second messenger) from ATP
  - cAMP activates protein kinases, which then cause cellular effects
- Many extracellular signal molecules work by increasing cyclic AMP content, and they do so by increasing the activity of ***adenylate cyclase*** rather than decreasing the activity of phosphodiesterase.
  - There are at least eight isoforms in mammals, most of which are regulated by both G proteins and Ca^{2+}.
- All receptors that act via cAMP are coupled to either a **stimulatory G protein (G_s)**, or to **inhibitory G protein (**
  - G_s- activates adenylyl cyclase and thereby increases cAMP concentration (target for ***cholera toxin***)
  - G_i- inhibits adenylyl cyclase, but it mainly acts by directly regulating ion channels rather than by decreasing cyclic AMP content (target for ***pertussis toxin***)



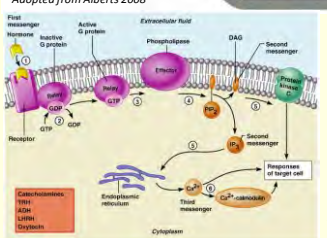
Adopted from Silverthorn 2010

The two branches of the inositol phospholipid pathway

- The activated receptor stimulates the plasma-membrane-bound enzyme *phospholipase C* via a G protein.
- Depending on the isoform of the enzyme, it may be activated by the α subunit of G_q as shown, by the βγ complex of another G protein, or by both. Two intracellular messenger molecules are produced when *$PI(4,5)P_2$* is hydrolyzed by the activated phospholipase C-β.
  - Inositol 1,4,5-trisphosphate (***IP_3***) – releases Ca^{2+}
  - Diacylglycerol (***DAG***) remains in the plasma membrane and, together with phosphatidylserine and Ca^{2+}, helps to activate the ***PKC*** (11 or more distinct isoforms, 4 activated by DAG) in mammals.



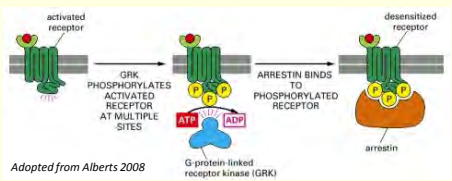
Adopted from Alberts 2008



Adopted from Silverthorn 2010

G-Protein-linked Receptor Desensitization Depends on Receptor Phosphorylation

- Target cells use a variety of mechanisms to *desensitize*, or *adapt,* when they are exposed to a high concentration of stimulus for a prolonged period
- Mechanisms that involve an alteration in GPL receptors themselves:

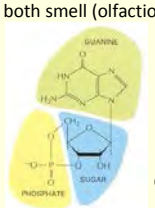


Adopted from Alberts 2008

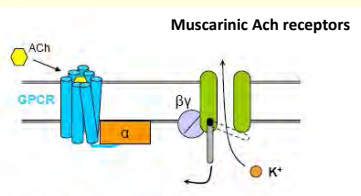
- These can desensitize in three general ways:
  1. They can become altered so that they can no longer interact with G proteins ***(receptor inactivation)***.
  2. They can be temporarily moved to the interior of the cell (internalized) so that they no longer have access to their ligand ***(receptor sequestration)***.
  3. They can be destroyed in lysosomes after internalization ***(receptor down-regulation)***.

Some G Proteins Directly Regulate Ion Channels

- G proteins do not act exclusively by regulating the activity of membrane-bound enzymes that alter the concentration of cyclic AMP or Ca^{2+} in the cytosol.
  - The α subunit of one type of G protein (called G_{12}), for example, activates a protein that converts a monomeric GTPase of the Rho family into its active form, which then alters the cytoskeleton.
  - In some other cases, G proteins directly activate or inactivate ion channels (Ach muscarinic receptors) in the plasma membrane of the target cell,
- Other trimeric G proteins regulate the activity of ion channels less directly, either by stimulating channel phosphorylation (by PKA,PKC, or CaM-kinase, for example) or by causing the production or destruction of cyclic nucletides that directly activate or inactivate ion channels . The cyclic-nucleotide-gated ion channels have a crucial role in both smell (olfaction) and vision.



cGMP

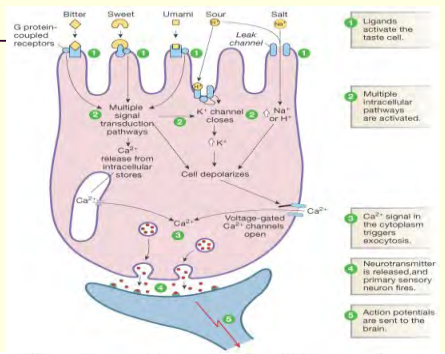


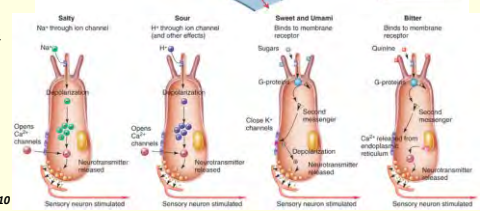
Examples of G-protein regulated mechanisms in cells

Perception of Taste

Points to remember:

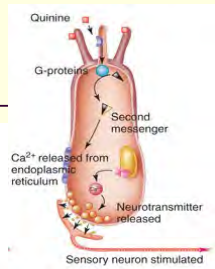
- There are multiple mechanisms for the detection of individual classes (sweet, sour, salty, umami etc.) of taste stimuli
- There is overlap among some of the transduction mechanisms across classes of stimuli
- A rise in intracellular [Ca^{2+}]inis a common point for all taste stimuli
- Salty & sour (acids):
- Act primarily at ion channels to depolarize taste cells
  - Sweet compounds, amino acids and bitter compounds:
- Act through ligand-gated channels & metabotropic receptors
  - There is NO regional tongue map for taste sensitivities
- Integration
  - Thalamus
  - Gustatory cortex
- "Specific hunger"





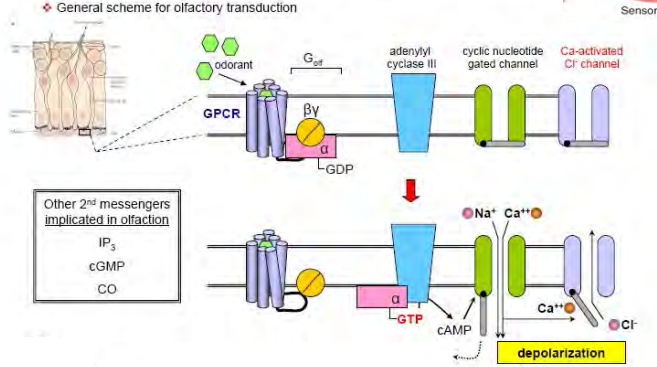
Adopted from Silverthorn 2010

Chemical Senses - Smell

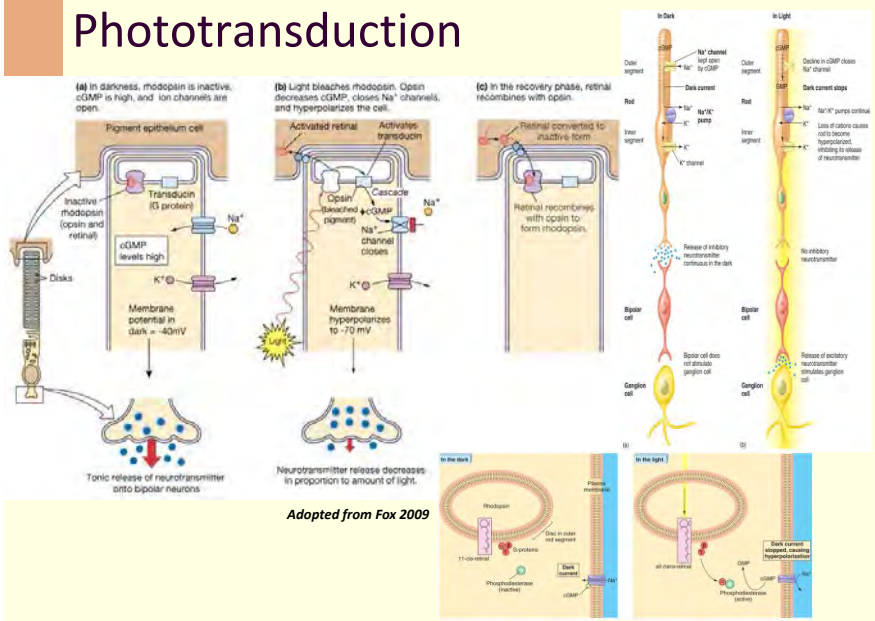


Olfactory Transduction

- Olfactory receptors
  - Encoded for by a large multigene family (at least ~1000 members)
  - All have same general structure but ↑ sequence diversity
    - G protein-coupled receptors (GPCRs): 7 transmembrane domains…
  - Diversity allows for identification of a huge variety of different chemical structures
- General scheme for olfactory transduction



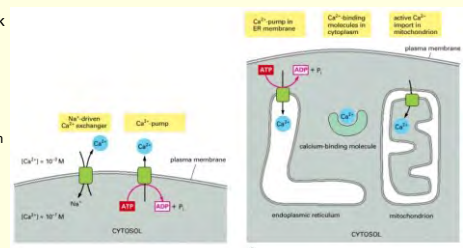
Phototransduction



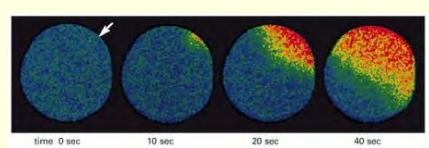
Adopted from Fox 2009

Ca^{2+} Functions as a Ubiquitous Intracellular Messenger

- Many extracellular signals induce an increase in cytosolic Ca^{2+} level, not just those that work via G proteins.
  - In egg cells, for example, a sudden rise in cytosolic Ca^{2+} upon fertilization by a sperm triggers a Ca^{2+} wave that is responsible for the onset of embryonic development
  - In muscle cells, Ca^{2+} triggers contraction, and in many secretory cells, including neurons, it triggers secretion
- Ca^{2+} can be used as a signal in this way because its concentration
  - in the cytosol is normally kept very low (~10^{-7} M),
  - in the extracellular fluid (~10^{-3} M)
  - in the ER is high (~10^{-3} M)
- Thus, there is a large gradient driving Ca^{2+} into the cytosol across both the plasma membrane and the ER membrane .
- When a signal transiently opens Ca^{2+} channels in either of these membranes,
  - increasing the local Ca^{2+} concentration by 10–20-fold
  - Three main types of Ca^{2+} channels (Ca_v,IP_3,RyR)

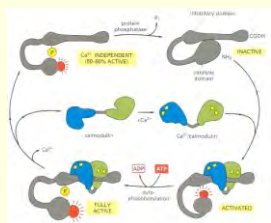


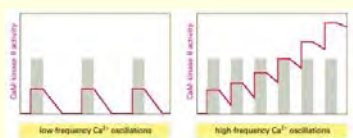
Adopted from Alberts 2008



Ca^{2+}/Calmodulin-dependent Protein Kinases (CaM-Kinases) Mediate Many of the Actions of Ca^{2+} in Animal Cells

- *Ca^{2+}-binding proteins* serve as transducers of the cytosolic Ca^{2+} signal.
  - *troponin C* in skeletal muscle cells
  - calmodulin found in all eucaryotic cells
- Many effects of Ca^{2+}, however, are more indirect and are mediated by phosphorylation catalyzed by a family of **Ca^{2+} /calmodulin-dependent kinases (*CaM-kinases*)**
- The best-studied example of such a *multifunctional CaM-kinase* is CaMK II, which is found in all animal cells but is especially enriched in the nervous system
  - can function as a molecular memory device, switching to an active state when exposed to CaCaM and then remaining active even after the Ca^{2+} signal has decayed
  - to act as a frequency decoder of Ca^{2+} oscillations
    - at different frequencies that mimic those observed in stimulated cells, the enzyme's activity increases steeply as a function of pulse frequency

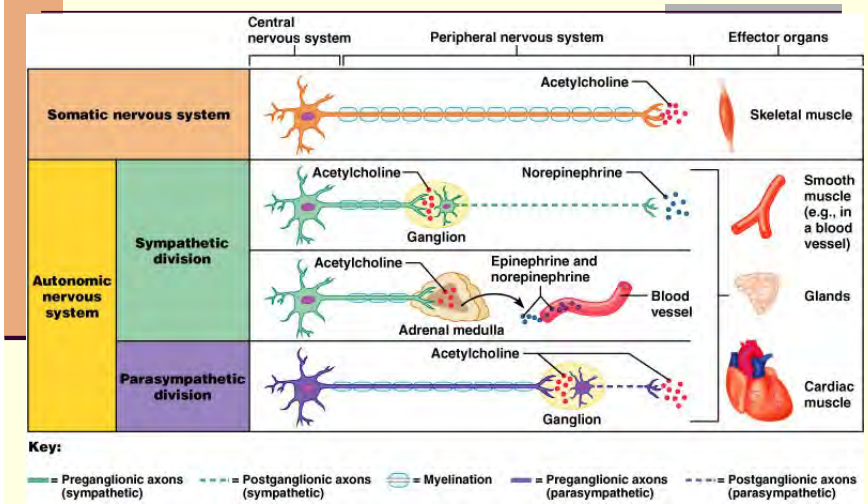




Adopted from Alberts 2008

Ligand-gated receptors

Neurotransmiter signalling



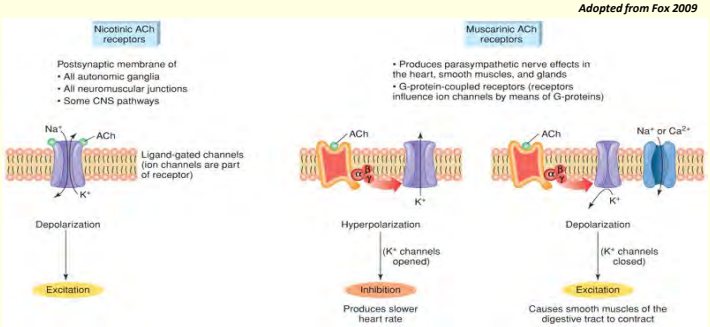
Adopted from Fox 2009

Acetylcholine synaptic transmission

Cholinergic Stimulation

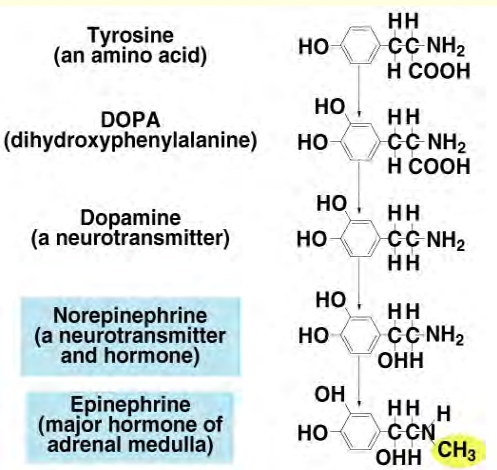
- ACh is used at all motor neuron synapses on skeletal muscle, all preganglionics, and Parasymp postganglionics
- Cholinergic receptors have 2 subtypes:
  - Nicotinic which is stimulated by nicotine; blocked by curare
  - And muscarinic which is stimulated by muscarine (from poisonous mushrooms); blocked by atropine

Adopted from Fox 2009



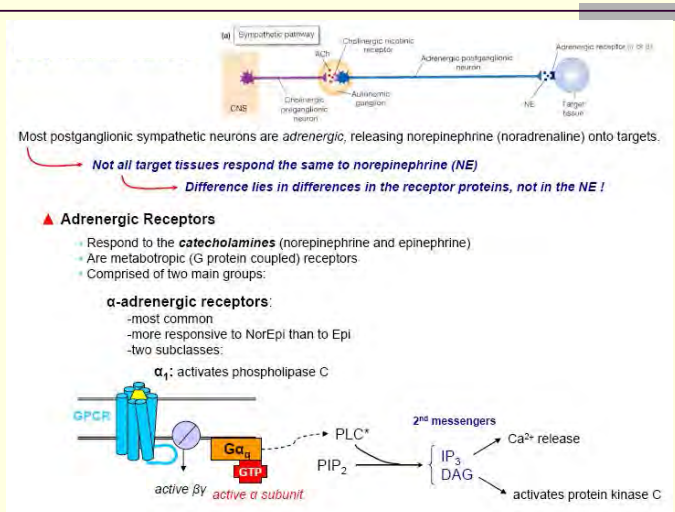
Adrenergic Synaptic Transmission

- Transmission at these synapses is called adrenergic:
  - NT released by most postganglionic sympathetic nerve fibers is NE.
  - Epi, released by the adrenal medulla is synthesized from the same precursor as NE.
- Collectively called catecholamines.



Adopted from Fox 2009

Adrenergic receptors



Most postganglionic sympathetic neurons are *adrenergic*, releasing norepinephrine (noradrenaline) onto targets.

Not all target tissues respond the same to norepinephrine (NE)

Difference lies in differences in the receptor proteins, not in the NE !

▲ **Adrenergic Receptors**

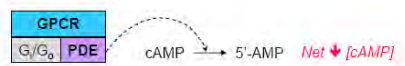
- Respond to the ***catecholamines*** (norepinephrine and epinephrine)
- Are metabotropic (G protein coupled) receptors
- Comprised of two main groups:

α-adrenergic receptors:
-most common
-more responsive to NorEpi than to Epi
-two subclasses:

α_1: activates phospholipase C

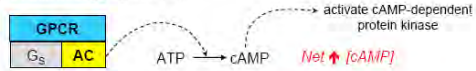
Adrenergic receptors

α_2: activates phosphodiesterase



β-adrenergic receptors
-two subclasses:

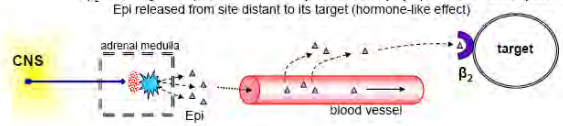
β_1: activates adenylyl cyclase (AC)



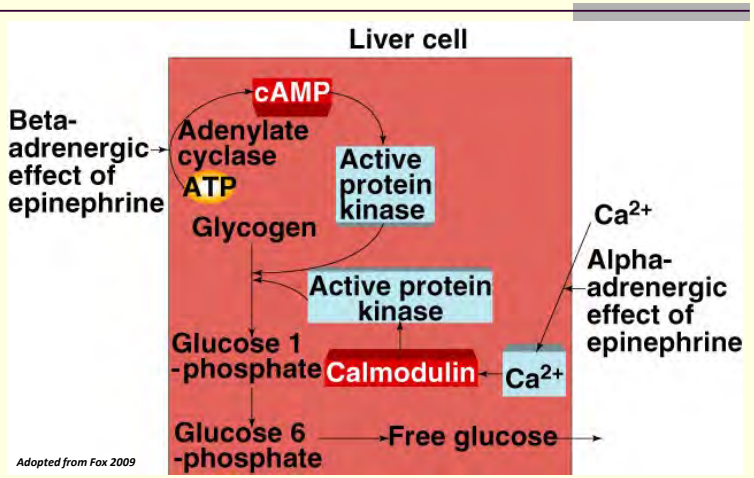
β_1: ~equally responsive to NorEpi and Epi

β_2: also activates adenylyl cyclase (AC); *see above*

β_2: - much more responsive to Epi than to NorEpi
- β_2-adrenergic receptors are not directly innervated by sympathetic nervous system
Epi released from site distant to its target (hormone-like effect)



Catecholamines can act through two 2nd messenger systems.



Enzyme-Linked Cell-Surface Receptors

Signaling through Enzyme-Linked Cell-Surface Receptors

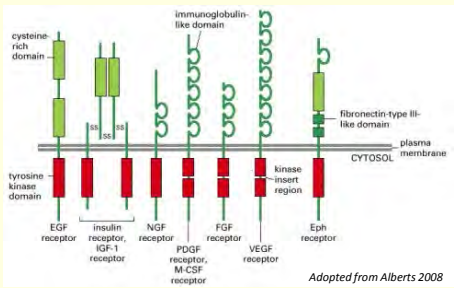
- Enzyme-linked receptors are a second major type of cell-surface receptor. They were recognized initially through their role in responses to extracellular signal proteins that promote the growth, proliferation, differentiation, or survival of cells in animal tissues. These signal proteins are often collectively called growth factors, and they usually act as local mediators (signaling molecules) at very low concentrations (about 10^{-9}-10^{-11} M). The responses to them are typically slow (on the order of hours) and usually require many intracellular signaling steps that eventually lead to changes in gene expression.
  - Enzyme-linked receptors have since been found also to mediate direct, rapid effects on the cytoskeleton, controlling the way a cell moves and changes its shape. The extracellular signals that induce these rapid responses are often not diffusible but are instead attached to surfaces over which the cell is crawling. Disorders of cell proliferation, differentiation, survival, and migration are fundamental events that can give rise to cancer, and abnormalities of signaling through enzyme-linked receptors have major roles in this class of disease.
- Like G-protein-linked receptors, enzyme-linked receptors are transmembrane proteins with their ligand-binding domain on the outer surface of the plasma membrane. Instead of having a cytosolic domain that associates with a trimeric G protein, however, their cytosolic domain either has an intrinsic enzyme activity or associates directly with an enzyme. Whereas a G-protein-linked receptors has seven transmembrane segments, each subunit of an enzyme-linked receptor usually has only one.

Signaling through Enzyme-Linked Cell-Surface Receptors

- There are five known classes of ***enzyme-linked receptors*** :
  - 1. receptor tyrosine kinases,
  - 2. tyrosine-kinase-associated receptors,
  - 3. receptor serine/threonine kinases,
  - 4. transmembrane guanylyl cyclases,
  - 5. histidine-kinase associated receptors.

1. Receptor Tyrosine Kinases

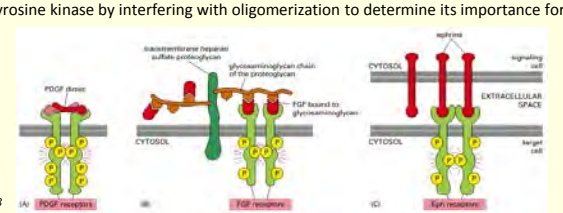
- Ligand binding to receptor tyrosine kinases induces the receptors to cross-phosphorylate their cytoplasmic domains on multiple tyrosines. The autophosphorylation activates the kinases, as well as producing a set of phosphotyrosines that then serve as docking sites for a set of intracellular signaling proteins, which bind via their SH2 (or PTB) domains. Some of the docked proteins serve as adaptors to couple the receptors to the small GTPase Ras, which, in turn, activates a cascade of serine/threonine phosphorylations that converge on a MAP-kinase, which relays the signal to the nucleus by phosphorylating gene regulatory proteins there. Ras can also activate another protein that docks on activated receptor tyrosine kinases—PI 3-kinase—which generates specific inositol phospholipids that serve as docking sites in the plasma membrane for signaling proteins with PH domains, including protein kinase B (PKB).



Adopted from Alberts 2008

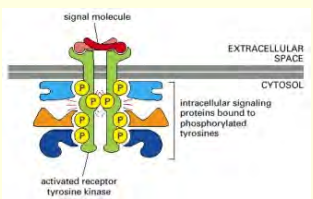
Receptor Tyrosine Kinases

- How does the binding of an extracellular ligand activate the kinase domain on the other side of the plasma membrane ?
  - the enzyme-linked receptors, two or more receptor chains come together in the membrane, forming a dimer or higher oligomer.
    - ***ligand binding induces the oligomerization.***
    - the oligomerization can occur before ligand binding, and the ligand causes a reorientation of the receptor chains in the membrane.
    - In either case, the rearrangement induced in cytosolic tails of the receptors initiates the intracellular signaling process. For receptor tyrosine kinases, the rearrangement enables the neighboring kinase domains of the receptor chains to cross-phosphorylate each other on multiple tyrosines, a process referred to as *autophosphorylation.*
- When the receptor chains are cross-linked, the kinase domains of adjacent receptors cross-phosphorylate each other, stimulating the kinase activity of the receptor and creating docking sites for intracellular proteins.
- Because of the requirement for receptor oligomerization, it is relatively easy to inactivate a specific receptor tyrosine kinase by interfering with oligomerization to determine its importance for a cell response

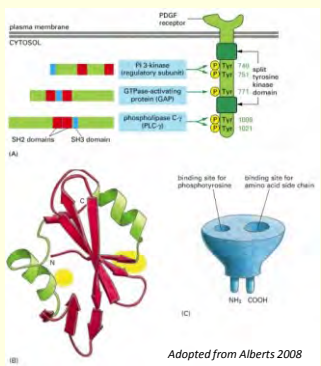


Adopted from Alberts 2008

Phosphorylated Tyrosines Serve as Docking Sites For Proteins With SH2 Domains



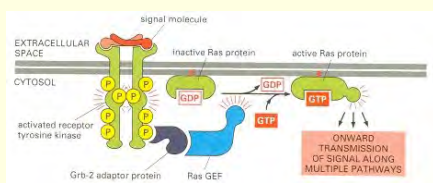
Adopted from Alberts 2008



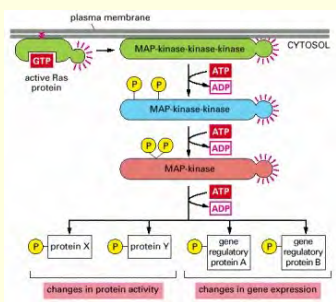
Adopted from Alberts 2008

Ras Is Activated by a Guanine Nucleotide Exchange Factor

- Some signaling proteins are composed almost entirely of SH2 and SH3 domains and function as adaptors to couple tyrosine-phosphorylated proteins to other proteins that do not have their own SH2 domains . Such adaptor proteins help to couple activated receptors to the important downstream signaling protein *Ras*
  - Most of the signaling proteins bound to the activated receptor are omitted for simplicity. The Grb-2 adaptor protein binds to a specific phosphotyrosine on the receptor and to the Ras guanine nucleotide exchange factor(GEF), which stimulates Ras to exchange its bound GDP for GTP. The activated Ras then activates several downstream signaling pathways

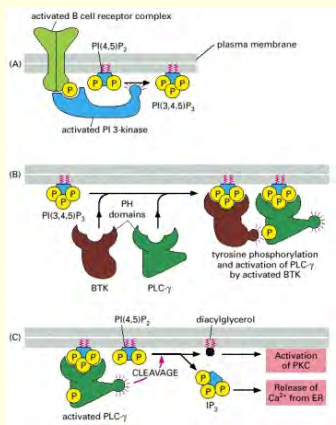


Adopted from Alberts 2008

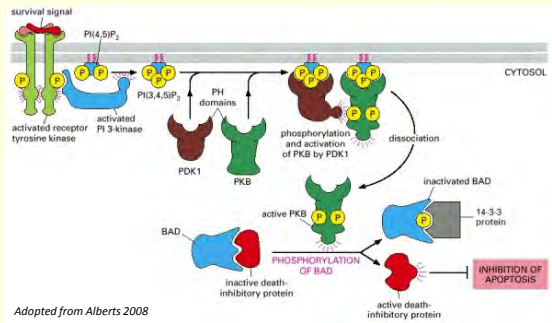


PI 3-Kinase Produces Inositol Phospholipid Docking Sites in the Plasma Membrane

- Extracellular signal proteins stimulate cells to divide, in part by activating the Ras- MAP-kinase pathway just discussed. If cells continually divided without growing, however, they would get progressively smaller and would eventually disappear. Thus, to proliferate, most cells need to be stimulated to enlarge (grow), as well as to divide. In some cases, one signal protein does both; in others one signal protein (a *mitogen)* mainly stimulates cell division, while another (a *growth factor)* mainly stimulates cell growth. One of the major intracellular signaling pathways leading to cell growth involves **phosphatidylinositol 3-kinase (PI 3-kinase)**. This kinase principally phosphorylates inositol phospholipids rather than proteins; it can be activated by receptor tyrosine kinases, as well as by many other types of cell-surface receptors, including some that are G-protein-linked.
- **Phosphatidylinositol** *(PI)* is unique among membrane lipids because it can undergo reversible phosphorylation at multiple sites to generate a variety of distinct inositol phospholipids. When activated, PI 3-kinase catalyzes the phosphorylation of inositol phospholipids at the 3 position of the inositol ring to generate lipids called *PI(3,4)*P_2 or *PI(3,4,5)*P_3 . The PI(3,4)P_2 and PI(3,4,5)P_3 then serve as docking sites for intracellular signaling proteins, bringing these proteins together into signaling complexes, which relay the signal into the cell from the cytosolic face of the plasma membrane.

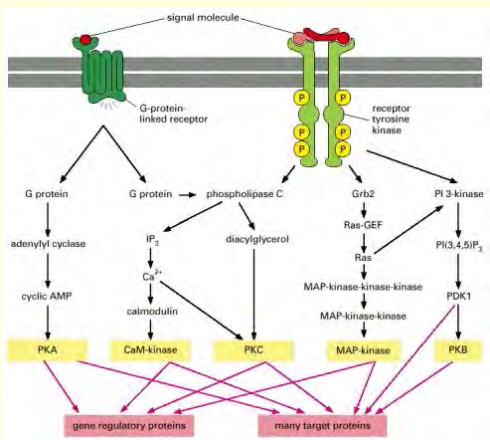


The PI 3-Kinase/Protein Kinase B Signaling Pathway Can Stimulate Cells to Survive and Grow



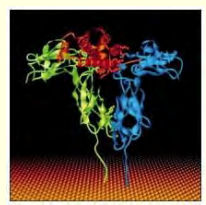
Adopted from Alberts 2008

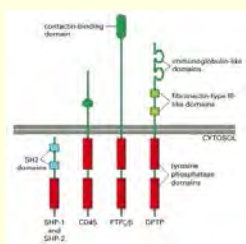
Five parallel intracellular signaling pathways activated by G-protein-linked receptors, receptor tyrosine kinases, or both



Adopted from Alberts 2008

2. Tyrosine-kinase-associated receptors

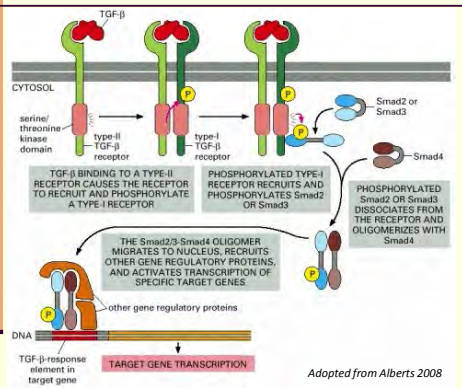




Adopted from Alberts 2008

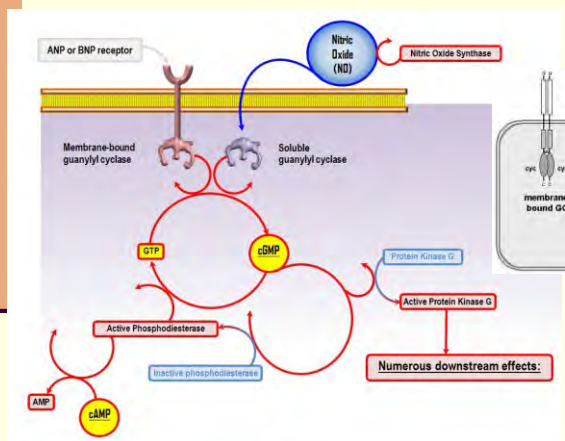
- Many cell-surface receptors depend on tyrosine phosphorylation for their activity and yet lack an obvious tyrosine kinase domain
- these receptors must oligomerize to function
- depend on various cytoplasmic tyrosine kinases for their action. These kinases include members of the Src family, which associate with many kinds of receptors, and the focal adhesion kinase(FAK), which associates with integrins at focal adhesions. The cytoplasmic tyrosine kinases then phosphorylate a variety of signaling proteins to relay the signal onward. The largest family of receptors in this class is the ***cytokine receptors*** family. When stimulated by ligand binding, these receptors activate Jak cytoplasmic tyrosine kinases, which phosphorylate STATs. The ***STATs*** then dimerize, migrate to the nucleus, and activate the transcription of specific genes.
  - Cytokine receptors constitute the largest and most diverse class of receptors that rely on cytoplasmic kinases to relay signals into the cell. They include receptors for many kinds of local mediators (collectively called cytokines), as well as receptors for hormones, such as growth hormone and prolactin. These receptors are stably associated with a class of cytoplasmic tyrosine kinases called *Jaks,* which activate latent gene regulatory proteins called *STATs*. The STAT proteins are normally inactive, being located at the cell surface; cytokine or hormone binding causes them to migrate to the nucleus and activate gene transcription.

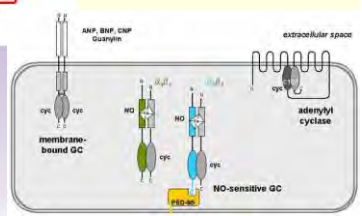
3. Receptor serine/threonine kinases



- Receptor serine/threonine kinases, which are activated by signaling proteins of the ***TGF-b superfamily***, act similarly: they directly phosphorylate and activate Smads, which then oligomerize with another Smad, migrate to the nucleus, and activate gene transcription.

4. Guanylate Cyclase receptors

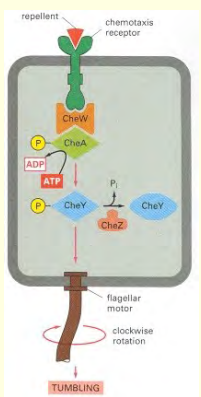




Affected proteins:

- cGNP-gated ion channels
- From cGMP-modulated phosphodiesterase

5. Histidine-Kinase-Associated Chemotaxis Receptors



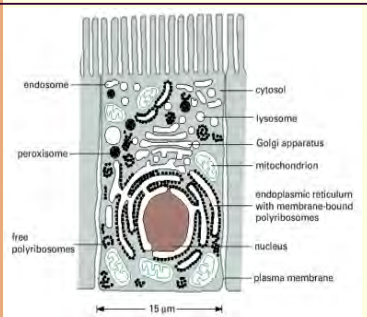
Adopted from Alberts 2008

- Bacterial chemotaxis is mediated by *histidine-kinase-associated chemotaxis receptors*. When activated by the binding of a repellent, the receptors stimulate their associated protein kinase to phosphorylate itself on histidine and then transfer that phosphate to a messenger protein, which relays the signal to the flagellar motor to alter the bacterium's swimming behavior. Attractants have the opposite effect on this kinase and therefore on swimming.

- The histidine kinase CheA is stably bound to the receptor via the adaptor protein CheW. The binding of a repellent increases the activity of the receptor, which stimulates CheA to phosphorylate itself on histidine. CheA quickly transfers its covalently bound, high-energy phosphate directly to CheY to generate CheY-phosphate, which then diffuses away, binds to the flagellar motor, and causes the motor to rotate clockwise, which results in tumbling. The binding of an attractant has the opposite effect: it decreases the activity of the receptors and therefore decreases the phosphorylation of CheA and CheY, which results in counterclockwise flagellar rotation and smooth swimming. CheZ accelerates the autodephosphorylation of CheY-phosphate, thereby inactivating it. Each of the phosphorylated intermediates decays in about 10 seconds, enabling the bacterium to respond very quickly to changes in its environment

Cell compartments, intracellular transport and molecular motors

The Compartmentalization of Cells

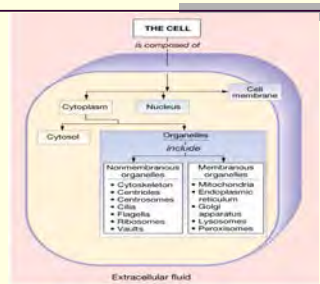


Adopted from Alberts 2008

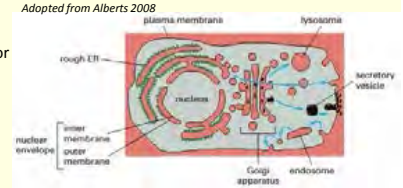
- Compartmentalization and inner membranes enables eukaryotic cells
  - to be 1000-10000 times larger than prokaryotes
  - to isolate specialized chemical processes in specific parts of the cell
  - to produce "packages" (vesicles) of chemical components that can be shuttled around the cell actively
- All eukaryotic cells have the same basic set of membrane-enclosed organelles
  - Intracellular membrane systems, however, do more for the cell than just provide increased membrane area: they create enclosed compartments that are separate from the cytosol, thus providing the cell with functionally specialized aqueous spaces.
  - Because the lipid bilayer of organelle membranes is impermeable to most hydrophilic molecules, the membrane of each organelle must contain membrane transport proteins that are responsible for the import and export of specific metabolites. Each organelle membrane must also have a mechanism for importing, and incorporating into the organelle, the specific proteins that make the organelle unique.
- Many vital biochemical processes take place in or on membrane surfaces.
  - Lipid metabolism is catalyzed mostly by membrane -bound enzymes.
  - Oxidative phosphorylation and photosynthesis both require a membrane to couple the transport of H^+ to the synthesis of ATP.

Cell organelles

- **Cytosol** – (54% of cell volume) fluid portion of the cytoplasm outside of the organells
- **Nucleus** - (6 % of cell volume)
- **Organelles** – (take up to 50 % of cell volume)
  - Membraneous – have characteristic positions in the cytosol
  - Nonmembraneous
- **Inclusions** – glycogen granules, lipid droplets, pigments
- Topologically equivalent spaces are shown in *red*. In principle, cycles of membrane budding and fusion permit the lumen of any of these organelles to communicate with any other and with the cell exterior by means of transport vesicles.

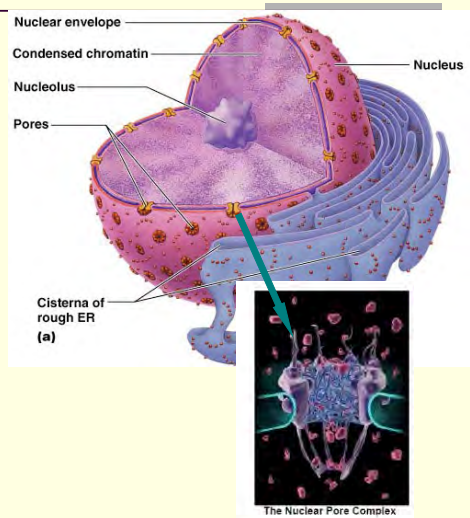


Adopted from Alberts 2008



Cell nucleus and genetic control

- **Nucleus**
  - most cells have single nucleus
  - contains genetic material
  - controls all cell processes:
    - Gene expression
    - Protein synthesis
    - Cell division
    - Cell death



Membrane-enclosed organelles

Membrane-enclosed organelles take up ~50% of the volume of eukaryotic cells:

- nucleus – genomic function
- endoplasmic reticulum – synthesis of lipids; on the border with the cytosol, synthesis of proteins destined for many organelles and the plasma membrane
- Golgi apparatus – modification, sorting, and packaging of proteins and lipids for specific intracellular destination (akin to a mail sort facility)
- lysosomes – degradation
- endosomes – sorting of endocytosed (engulfed) material by the cell
- peroxisomes – oxidation of toxic species
- mitochondria, chloroplasts – energy conversion

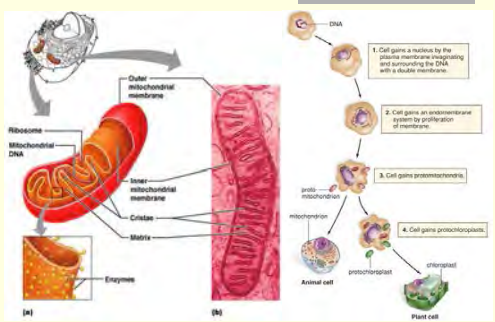
Cells contain 10 10 protein molecules that are constantly being synthesized and degraded.

Proteins are synthesized in the cytosol, but not all proteins remain there and many must be transported to the appropriate compartment

Mitochondria –power plants of the cell

Function:

- Majority of ATP production
- O2 consumption
- CO2 production
- Contains enzymes active in Krebs cycle and oxidative phosphorylation



Complex organelles – contain their own DNA and RNA and are able to reproduce themselves

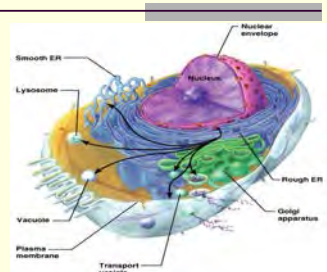
Endomembrane System

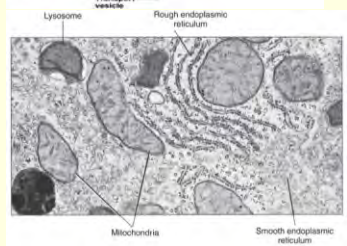
Rough endoplasmic reticulum (Rough ER)

- ***Structure:*** Extensive membranous network of flattened sacs. It encloses a space that is continuous throughout the organelle and with the space between two nuclear envelopes. It has ribosomes attached to its cytosolic surface.
  - ***Function:*** Protein and phospholipids synthesis. Proteins synthetized on the attached ribosomes then enter lumen from which they are transported to their target site.

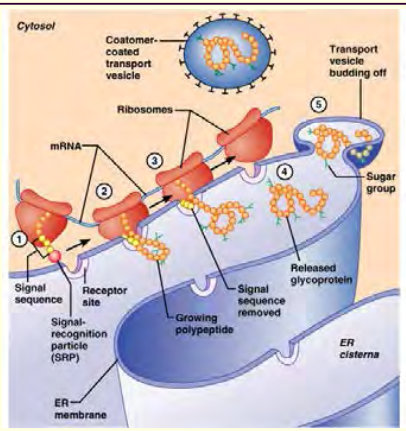
Smooth endoplasmic reticulum (Smooth ER)

- ***Structure:*** Highly branched tubular network without ribosomes, but may be continuous with rough ER.
- ***Function:***
  - Fatty acid, cholesterol and steroid hormone synthesis.
  - Stores and releases calcium.
  - Detoxification (liver, kidney) – ***drug tolerance***.
  - Breakdown glycogen



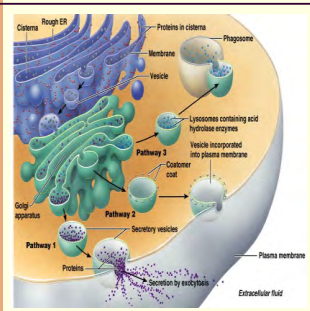


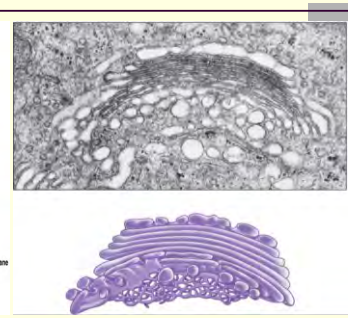
Signal Mechanism of Protein Synthesis



- Proteins to be secreted are made in ribosomes of rough ER
  - Contain a leader sequence of 30+ hydrophobic amino acids that directs such proteins to enter cisternae of ER
    - Where leader sequence is removed; protein is modified

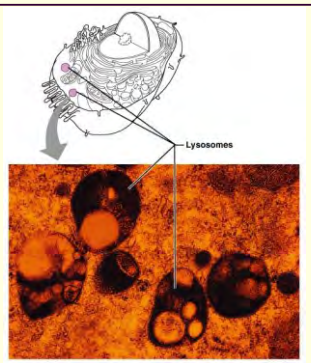
Golgi Apparatus





- ***Structure:*** Series of apposed flattened sacs associated with numerous vesicles. Golgi is located in the central part of a cell near nucleus.
- ***Function:*** Trafficking of cellular proteins – modification, sorting, concentrating, and packaging

Lysosomes - cellular stomachs



Peroxisomes

- Are vesicle-like organelles containing oxidative enzymes
  - Involved in detoxification in liver - removal of hydrogen and H_2O_2

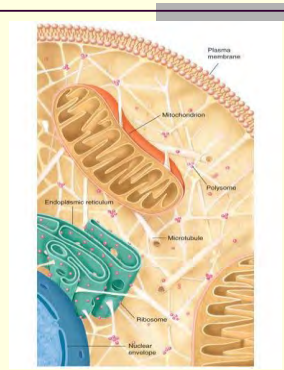
- Are vesicle-like organelles containing digestive enzymes and matter being digested
  - Involved in recycling cell components
  - Involved in programmed cell death
- **Functions:**
  - digesting particles
  - degrading organelles
  - metabolism – breaking down glycogen
  - breaking down tissues
  - breaking down bone

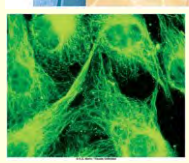
- **Genetic illness:**
- **Tay-Sachs disease** is a fatal genetic lipid storage disorder caused by insufficient activity of an enzyme called *beta-hexosaminidase A* that catalyzes the biodegradation of acidic fatty materials
  - http://www.ninds.nih.gov/disorders/taysachs/taysachs.htm
- **Gaucher's disease** - an inherited metabolic disorder in which harmful quantities of a fatty substance called *glucocerebroside* accumulate in the spleen, liver, lungs, bone marrow, and sometimes in the brain . It is caused by a deficiency of an enzyme called *glucocerebrosidase*.
  - http://www.ninds.nih.gov/disorders/gauchers/gauchers.htm

Organelles

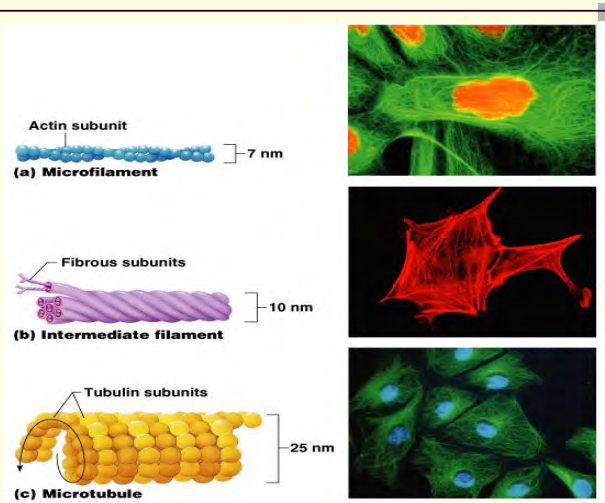
- Nonmembraneous
  - **ribosomes** – protein factories
  - **endosomes** – further trafficking of cellular proteins
  - **cytoskeleton** – maintain cell shape and cell movement

- **Cytoskeleton** is a latticework of microfilaments and microtubules filling cytoplasm
  - Gives cell its shape and structure
  - Forms tracks upon which things are transported around cell



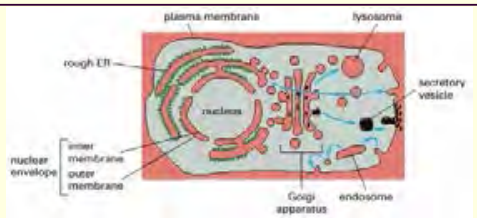


Cytoskeleton - maintain cell shape and cell movement



Cell compartments and intracellular transport

Topology of cell compartments



- Topologically equivalent spaces are shown in *red*. In principle, cycles of membrane budding and fusion permit the lumen of any of these organelles to communicate with any other and with the cell exterior by means of transport vesicles.

- Cells contain 10^{10} - 10^{12} protein molecules that are constantly being synthesized and degraded
- Proteins are synthesized in the cytosol, but not all proteins remain there and many must be transported to the appropriate compartment

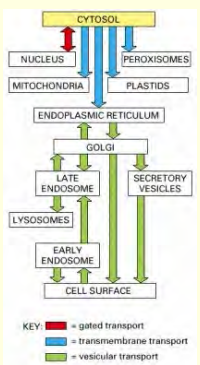
Most Membrane-enclosed Organelles Cannot Be Constructed From Scratch: They Require Information in the Organelle Itself

- When a cell reproduces by division, it has to duplicate its membrane-enclosed organelles. In general, cells do this by enlarging the existing organelles by incorporating new molecules into them; the enlarged organelles then divide and are distributed to the two daughter cells. Thus, each daughter cell inherits from its mother a complete set of specialized cell membranes. This inheritance is essential because a cell could not make such membranes from scratch.
- It seems that the information required to construct a membrane-enclosed organelles does not reside exclusively in the DNA that specifies the organelles' proteins. *Epigenetic* information in the form of at least one distinct protein that preexists in the organelles membrane is also required, and this information is passed from parent cell to progeny cell in the form of the organelles itself. Presumably, such information is essential for the propagation of the cell's compartmental organization, just as the information in DNA is essential for the propagation of the cell's nucleotide and amino acid sequences.
- During cell division, organelles such as the ER and mitochondria are distributed intact to each daughter cell. These organelles contain information that is required for their construction.

Proteins Can Move Between Compartments in Different Ways

- All proteins begin being synthesized on ribosomes in the cytosol, except for the few that are synthesized on the ribosomes of mitochondria. Their subsequent fate depends on their amino acid sequence, which can contain sorting signals that direct their delivery to locations outside the cytosol. Most proteins do not have a sorting signal and consequently remain in the cytosol as permanent residents. Many others, however, have specific sorting signals that direct their transport from cytosol into the nucleus, the ER, mitochondria, plastids, or peroxizomes; sorting signals can also direct the transport of proteins from the ER to other destinations in the cell.

- To understand the general principles by which sorting signals operate, it is important to distinguish three fundamentally different ways by which proteins move from one compartment to another.

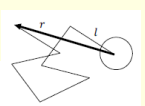


Protein transport

Transport by diffusion

- Molecules in the cell move rapidly by diffusion
- Due to thermal motion, the particle on average makes a random jump of length every units of time. The jump is random in the radial direction. This is called a ***random walk***

$$\langle r_n^2 \rangle = nl^2 = \frac{t}{\tau} l^2$$



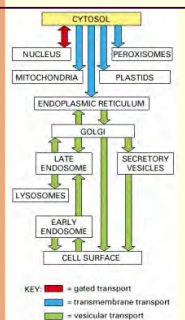
- r_n^2 – ***mean squared displacement.*** It tracks the average squared distance of a particle at time zero from its random location at time.
- This movement is called ***Brownian motion*** and is a kind of ***diffusive*** process.
- We can define the ***diffusion constant D***

$$D = \frac{r^2}{6t} \longrightarrow \langle r_n^2 \rangle = 6Dt$$

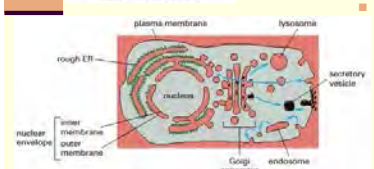
Active transport

- In reality, proteins do not move by simple diffusion
- **Free energy transduction** is used to power **active transport** of molecules
- Proteins are selectively transported to different parts of the eukaryotic cell by sorting
- Sorting includes **three basic mechanisms** by which proteins can move selectively to different parts of the cell

Proteins Can Move Between Compartments in Three Different Ways



- Proteins can move from one compartment to another by **gated transport** ***(red)***, **transmembrane transport** ***(blue)***, or **vesicular transport** ***(green)***. The signals that direct a given protein's movement through the system, and thereby determine its eventual location in the cell, are contained in each protein's amino acid sequence. At each intermediate station *(boxes)*, a decision is made as to whether the protein is to be retained in that compartment or transported further. In principle, a signal could be required for either retention in or exit from a compartment.



- **1** Gated transport – **transport via large openings** (the nuclear pore complexes) that allow **passage of folded proteins**. The protein traffic between the cytosol and nucleus occurs between topologically equivalent spaces, which are in continuity through the nuclear pore complexes.
- **2** Transmembrane transport - membrane-bound ***protein translocators*** directly transport **unfolded** specific proteins **across a membrane** from the cytosol into a space that is topologically distinct.

3 Vesicular transport - **membrane-enclosed transport** intermediates—which may be small, spherical transport vesicles or larger, irregularly shaped organelle fragments - ferry proteins from one compartment to another.

Signal Sequences and Signal Patches Direct Proteins to the Correct Cellular Address

Protein synthesis

Start of a protein: synthesis takes place

- in the cytosol on free ribosomes ⟹ proteins released to the cytosol
- in the cytosolic side of the rough ER on membrane-bound ribosomes ⟹ proteins enter ER

Many proteins stay in the cytosol, but for the others they must carry *signal sequences* at either the N or C terminal, or both, to direct to which compartment they go

- recognition of signal sequences by pores and transport proteins
- often removed from the protein after it has reached its final destination

(A) The signal resides in a single discrete stretch of amino acid sequence, called a ***signal sequence***, that is exposed in the folded protein. Signal sequences often occur at the end of the polypeptide chain (as shown), but they can also be located internally.

(B) A signal patch can be formed by the juxtaposition of amino acid from regions that are physically separated before the protein folds (as shown). Alternatively, separate patches on the surface of the folded protein that are spaced a fixed distance apart can form the signal.

Proteins released to the cytosol

Proteins entering the nucleus

- ***Incoming:*** histones, polymerases, regulatory factors
- ***Outgoing:*** rRNA, mRNA
- **Nuclear pores** are giant protein gateways that cross the nuclear envelope and allow selective passage of proteins destined for the nucleus
  - ~50 proteins
  - unstructured, entangled protein strands in the middle, like a kelp bed sieving property
  - 5-10 kDa molecules can pass through simple diffusion, larger requires active transport
  - ***nuclear transport receptor proteins*** bind to the nuclear signal sequence on cytosolic proteins and navigate them through this tangle
  - requires hydrolysis of GTP
  - proteins are transported in fully folded form

Proteins entering the mitochondria and chloroplasts

Though mitochondria and chloroplasts have their own genome, many of the genes for the

required proteins have evolved into the nuclear DNA, and thus these proteins must be imported

- signal sequence binds to receptors on the outer membrane of the organelle
- receptors diffuse laterally in the membrane until finding a contact site
- protein unfolds and passes through the contact site, to the interior of the organelle
- chaperones inside the organelle help pull the protein through and refold it

Proteins entering the ER

Proteins entering the ER

- Proteins that are eventually sent to the Golgi apparatus, endosomes, lysosomes, and cell surface all must first enter the ER
- Proteins can diffuse from the cytosol to the ER surface, but most are synthesized at the ER with membrane-bound ribosomes
  - ER signal sequence directs a ribosome to attach to the ER by a binding mechanism to a translocation channel
  - other ribosomes bind to the first membrane-attached ribosome, forming a polyribosome
- Two kinds of proteins can be synthesized by membrane-bound ribosomes:
  - soluble proteins are released to the interior of the ER (lumen)
  - transmembrane proteins are left inserted into the membrane of the ER by the translocation channel
    - hydrophobic start and stop transfer sequences tells the channel how to insert the protein in the membrane
    - orientation with respect to the membrane is subsequently fixed because flipping is so kinetically slow

Vesicular transport

- Once in the ER, proteins can be sent to many different intracellular and extracellular locations, but in all cases transport occurs by vesicles
- ER ⟼ Golgi apparatus ⟼ "-somes", plasma membrane, or extracellular space

Many proteins are selectively retained in the compartments in which they function

- The KDEL retrieval pathway only partly explains how ER resident proteins are maintained in the ER. As expected, cells that express genetically modified ER resident proteins, from which the KDEL sequence has been experimentally removed, secrete these proteins.
  - But secretion occurs at a much slower rate than for a normal secretory protein. It seems that ER resident proteins are anchored in the ER by a mechanism that is independent of their KDEL signal and that only those proteins that escape retention are captured and returned via the KDEL receptor.
  - A suggested mechanism of retention is that ER resident proteins bind to one another, thus forming complexes that are too big to enter transport vesicles. Because ER resident proteins are present in the ER at very high concentrations (estimated to be millimolar), relatively low-affinity interactions would suffice to have most of the proteins tied up in such complexes.
- Aggregation of proteins that function in the same compartment—called ***kin recognition***—is a general mechanism that compartments use to organize and retain their resident proteins. Golgi enzymes that function together, for example, also bind to each other and are thereby restrained from entering transport vesicles.

Transport from the ER

Vesicular transport

- Once in the ER, proteins can be sent to many different intracellular and extracellular locations, but in all cases transport occurs by vesicles
- ER ⟼ Golgi apparatus ⟼ "-somes", plasma membrane, or extracellular space

Basic features of transport

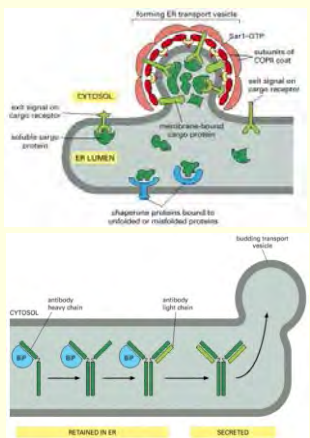
- **Specificity:** Vesicles carry specific cargo and drop it off at specific locations ⟼ events during packaging of materials and in vesicles interacting with other molecules
- Proteins involved in the formation and loading of vesicles
  - **clathrin** is a protein that self-assembles into basket-like cages to help bud off and stabilize a small vesicle
  - **dynamin** helps pinch off the end of the budding vesicle by constraining the "neck"
  - **transmembrane receptors** bind specific cargo along the inner vesicle membrane
  - **adaptins** bind and help assemble specific kinds of receptors for pinching off with clathrin
- How does a vesicle find the correct destination?
  - **Rab** proteins on the vesicle surface are recognized and bound by complementary cytosolic tethering proteins in the destination organelle
  - **SNARE** proteins on both the vesicle and organelle also recognize each other, wrapping around like a twist-tie and pulling the vesicle close to the destination organelle
- Vesicle fusion occurs with the help of proteins that assemble at the fusion site

Pathways of transport from the ER

- Proteins that are synthesized and remain in the cytosol are typically not post-translationally modified
- Proteins that enter the ER, however, are covalently modified:
  - disulfide bonds are formed used to increase stability of proteins that might need to be released to the harsh extracellular environment
  - glycosylation begins sugars help prevent degradation, sometimes assist folding, and can be recognition or transport signals
- Typically glycosylation occurs in chunks of sugars that are attached to the amino acid asparagine
- Glycosylation continues in the Golgi apparatus

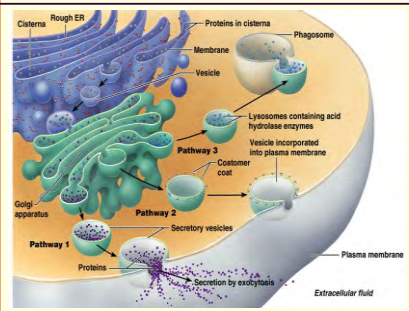
Only Proteins That Are Properly Folded and Assembled Can Leave the ER

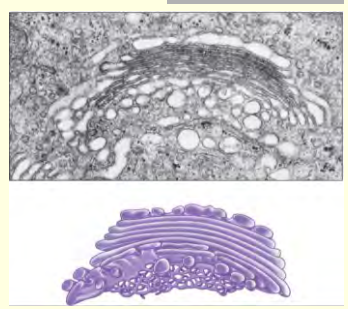
- The pathway from the ER to the cell surface involves many sorting steps, which continually select membrane and soluble luminal proteins for packaging and transport—in vesicles or organelle fragments that bud from the ER and Golgi apparatus.
- Exit from the ER serves as a quality-control mechanism for protein folding and assembly
  - unfolded proteins or multimeric structures not properly assembled are not allowed to exit
  - chaperones in the ER help fold proteins correctly so they can exit
  - proteins that don't fold correctly are eventually degraded in the ER
  - if too many unfolded proteins are present, the unfolded protein response program causes the production or more ER and its components
- Protein-containing vesicles exit the ER and fuse with the Golgi apparatus
  - vesicles enter and fuse on the ***cis*** face
  - vesicles bud off and exit with cargo on the ***trans*** face



Role of the Golgi Apparatus

Trafficking of cellular proteins – modification, sorting, concentrating, and packaging

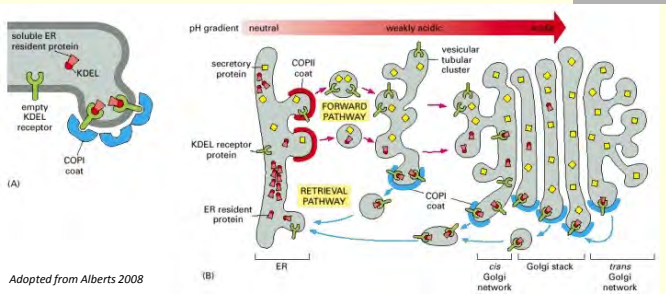




During their subsequent transport, from the ER to the Golgi apparatus and from the Golgi apparatus to the cell surface and elsewhere, proteins pass through a series of compartments, where they are successively modified.

- Transfer from one compartment to the next involves a delicate balance between **forward** and **backward (retrieval) transport pathways**.
- Some transport vesicles select cargo molecules and move them to the next compartment in the pathway, while others retrieve escaped proteins and return them to a previous compartment where they normally function.

The Retrieval Pathway to the ER Uses Sorting Signals

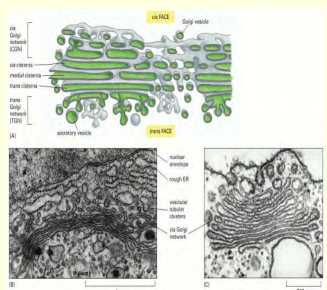


Adopted from Alberts 2008

- The retrieval pathway for returning escaped proteins back to the ER depends on **ER *retrieval signals***. Resident ER membrane proteins, for example, contain signals that bind directly to **COPI** coats and are thus packaged into COPI-coated transport vesicles for retrograde delivery to the ER.
  - The best-characterized signal of this type consists of two lysines, followed by any two other amino acids, at the extreme C-terminal end of the ER membrane protein. It is called a ***KKXX sequence***, based on the single-letter amino acid code.
- Soluble ER resident proteins, such as BiP, also contain a short retrieval signal at their C-terminal end, but it is different: it consists of a Lys-Asp-Glu-Leu or similar sequence. If this signal (called the *KDEL sequence*) is removed from BiP by genetic engineering, the protein is slowly secreted from the cell. If the signal is transferred to a protein that is normally secreted, the protein is now efficiently returned to the ER, where it accumulates.

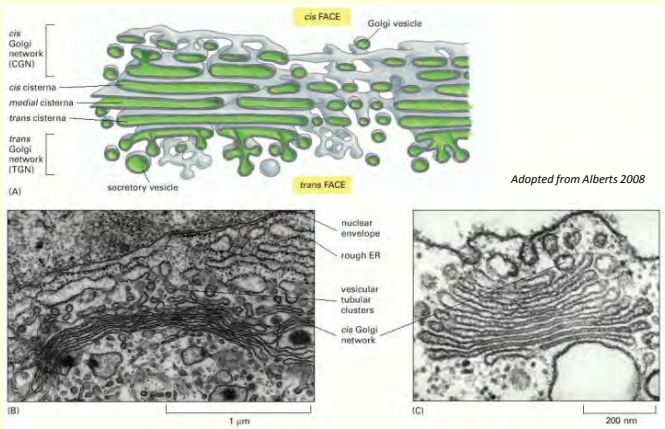
In the Golgi apparatus

- Protein-containing vesicles exit the ER and fuse with the Golgi apparatus
  - vesicles enter and fuse on the ***cis*** face
  - vesicles bud off and exit with cargo on the ***trans*** face
- proteins are further covalently modified, notably by addition of complex oligosaccharide groups
- proteins bud off in transport vesicles destined for specific locations
- location in the Golgi apparatus where budding occurs determines what kinds of modifications are made and to where the proteins are sent
- Secretion pathways for protein-containing vesicles
  - **default pathway** ⟼ no signal required, to the extracellular space
  - **constitutive exocytosis pathway** ⟼ contents used to supply plasma membrane with proteins and lipids
  - **regulated exocytosis pathway** ⟼ vesicles wait at the cell membrane for release triggered by changes in pH, ion concentration, or binding proteins



Adopted from Alberts 2008

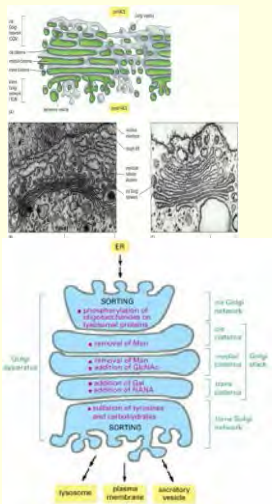
The Golgi Apparatus Consists of an Ordered Series of Compartments



Adopted from Alberts 2008

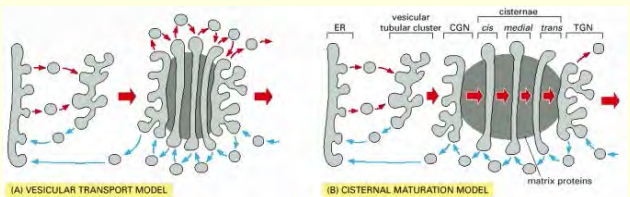
The Golgi Apparatus Consists of an Ordered Series of Compartments

- Proteins exported from the ER enter the first of the Golgi processing compartments (the ***cis*** Golgi compartment), after having passed through the *cis* Golgi network. They then move to the next compartment (the ***medial*** compartment, consisting of the central cisternae of the stack) and finally to the ***trans*** compartment, where glycosylation is completed. The lumen of the *trans* compartment is thought to be continuous with the *trans* Golgi network, where proteins are segregated into different transport packages and dispatched to their final destinations—the plasma membrane, lysosomes, or secretory vesicles.
- The oligosacharide processing steps occur in a correspondingly organized sequence in the Golgi stack, with each cisterna containing a characteristic abundance of processing enzymes.
- Proteins are modified in successive stages as they move from cisterna to cisterna across the stack, so that the stack forms a multistage processing unit. This compartmentalization might seem unnecessary, since each oligosacharide processing enzyme can accept a glycoprotein as a substrate only after it has been properly processed by the preceding enzyme. Nonetheless, it is clear that processing occurs in a spatial as well as a biochemical sequence: enzymes catalyzing early processing steps are concentrated in the cisternae toward the *cis* face of the Golgi stack, whereas enzymes catalyzing later processing steps are concentrated in the cisternae toward the *trans* face.



Adopted from Alberts 2008

Transport Through the Golgi Apparatus May Occur by Vesicular Transport or Cisternal Maturation



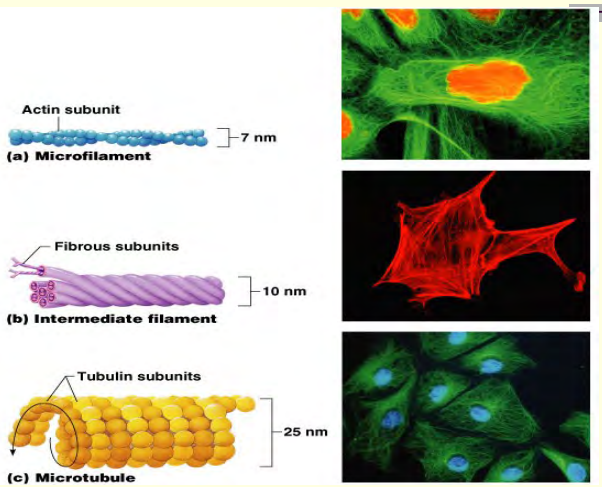
Adopted from Alberts 2008

- It is likely that the transport through the Golgi apparatus in the forward direction *(red arrows)* involves elements of both of the views represented here.
- (A) In the ***vesicular transport model***, Golgi cisternae are static organelles, which contain a characteristic complement of resident enzymes. The passing of molecules through the Golgi is accomplished by forward-moving transport vesicles, which bud from one cisterna and fuse with the next in a *cis*-to-*trans* direction.
- (B) According to the alternative ***cisternal maturation model***, each Golgi cisterna matures as it migrates outwards through a stack. At each stage, the Golgi resident proteins that are carried forward in a cisterna are moved backward to an earlier compartment in COPI-coated vesicles. When a newly formed cisterna moves around to a *medial* position, for example, "left-over" *cis* Golgi enzymes would be extracted and transported backward to a new cis cisterna behind. Likewise, the *medial* enzymes would be received by retrograde transport from the cisternae just ahead. In this way, a *cis* cisterna would mature to a *medial* cisterna as it moves.

Matrix Proteins Form a Dynamic Scaffold That Helps Organize the Apparatus

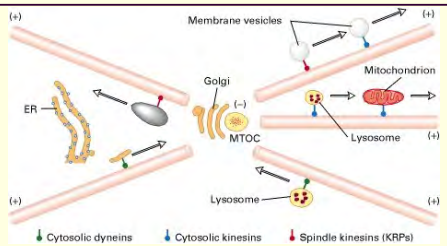
- The unique architecture of the Golgi apparatus depends on both the microtubule cytoskeleton, and cytoplasmic Golgi matrix proteins, which form a scaffold between adjacent cisternae and give the Golgi stack its structural integrity.
- Some of the matrix proteins form long, filamentous tethers that are thought to help retain Golgi transport vesicles close to the organelle. When the cell prepares to divide, mitotic protein kinases phosphorylate the Golgi matrix proteins, causing the Golgi apparatus to fragment and disperse throughout the cytosol. During disassembly, Golgi enzymes are returned in vesicles to the ER, while other Golgi fragments are distributed to the two daughter cells. There, the matrix proteins are dephosphorylated, leading to the reassembly of the Golgi apparatus.
- Remarkably, the Golgi matrix proteins can assemble into appropriately localized stacks near the centrosome even when Golgi membrane proteins are experimentally prevented from leaving the ER. This observation suggests that the matrix proteins are largely responsible for both the structure and location of the Golgi apparatus.

Cytoskeleton - maintains cell shape and enables cell movement



Molecular Motors
Intracellular transport of vesicles

How do vesicles move fast from one part of the cell to another?



Adapted from N. Hirokawa, 1998, *Science* **279**:519

- Vesicles do not simply diffuse around, but are actively transported to other parts of the cell
- ***Motor proteins*** "walk" along actin filaments and microtubules in the cell cytoskeleton, pulling attached vesicles
  - requires ATP hydrolysis
  - conformational changes due to ATP binding and hydrolysis produce net movement
  - proteins that walk along microtubules are ***kinesins*** (**outward** from the centrosome) and ***dyneins*** (**inward**)

Molecular Motors
Intracellular transport of vesicles

- Perhaps the most fascinating proteins that associate with the cytoskeleton are the **molecular motors** called motor proteins. These remarkable proteins bind to a polarized cytoskeletal filament and use the energy derived from repeated cycles of ATP hydrolysis to move steadily along it.
  - Dozens of different motor proteins coexist in every eukaryotic cell. They differ in the type of filament they bind to (either actin or microtubules), the direction in which they move along the filament, and the "cargo" they carry.
  - Many motor proteins carry membrane-enclosed organelles—such as mitochondria, Golgi stacks, or secretory vesicles—to their appropriate locations in the cell. Other motor proteins cause cytoskeletal filaments to slide against each other, generating the force that drives such phenomena as muscle contraction, ciliary beating, and cell division.
- Cytoskeletal motor proteins that move unidirectionally along an oriented polymer track are reminiscent of some other proteins and protein complexes such as DNA and RNA polymerases, helicases, and ribosomes. All of these have the ability to use chemical energy to propel themselves along a linear track, with the direction of sliding dependent on the structural polarity of the track. All of them generate motion by coupling nucleoside triphosphate hydrolysis to a large-scale conformational change in a protein.

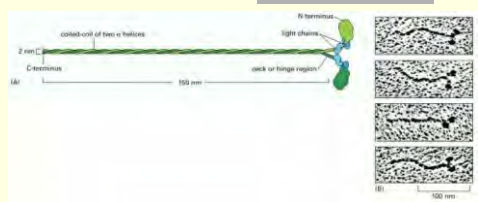
Functional Classes of Microtubule Motor Proteins

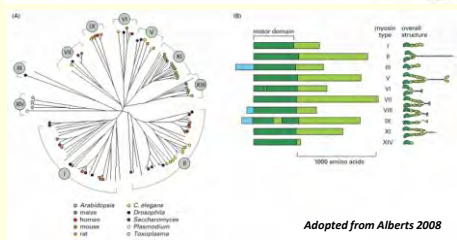
Class	Members	Cargo	Direction of Movement*
Cytosolic kinesins	Kinesin, Unc-104	Cytosolic vesicles	(+)
Spindle kinesins†	Ncd/KAR3, BimC/Eg5, CENP-E	Spindle and astral MTs, centrosomes, kinetochores	(+) or (−)
Cytosolic dyneins	Cytoplasmic dynein	Cytosolic vesicles, kinetochores during mitosis and meiosis	(−)
Axonemal dyneins	Outer-arm dyneins, inner-arm dyneins‡	A tubule of doublet microtubules in cilia and flagella	(−)

Adopted from NCBI Bookshelf: Lodish H, Berk A, Zipursky SL, et al. Molecular Cell Biology. 4th edition. New York: W. H. Freeman; 2000.

Actin-based Motor Proteins Are Members of the Myosin Superfamily

- The first motor protein identified was skeletal muscle **myosin**
  - responsible for generating the force for muscle contraction.
  - *myosin II* is an elongated protein that is formed from two heavy chains and two copies of each of two light chains.
- movement speed about **0.2 to 60 µm/sec** for myosins
- It was initially thought that myosin was present only in muscle, but in the 1970's, researchers found that a similar two-headed myosin protein was also present in non muscle cells, including protozoan cells. At about the same time, other researchers found a myosin in the freshwater amoeba
  - Subsequently, many other myosin types were discovered.
  - Structure: The heavy chains generally start with a recognizable myosin motor domain at the N-terminus, and then diverge widely with a variety of C-terminal tail domain.
  - The new types of myosins include a number of one-headed and two-headed varieties that are approximately equally related to myosin I and myosin II.
  - The myosin tails (and the tails of motor proteins generally) have apparently diversified during evolution to permit the proteins to dimerize with other subunits and to interact with different cargoes.

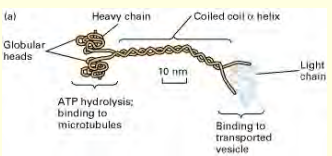




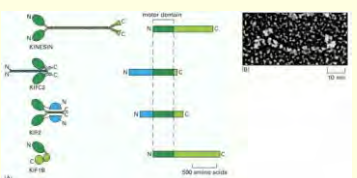
Adopted from Alberts 2008

There Are Two Types of Microtubule Motor Proteins: **Kinesins** and Dyneins

- ***Kinesin*** is a motor protein that moves cargo **outwards** along microtubules. It was first identified in the giant axon of the squid, where it carries membrane-enclosed organelles away from the neuronal cell body toward the axon terminal by walking toward the plus end of microtubule.
  - Kinesin is similar structurally to myosin II in having two heavy chains and two light chains per active motor, two globular head motor domains, and an elongated coiled-coil responsible for heavy chain dimerization. Like myosin, kinesin is a member of a large protein superfamily, for which the motor domain is the only common element.
- movement speed about **0.02 to 2 µm/sec**
- There are at least ten families of kinesin **-related proteins**, or **KRPs**, in the kinesin superfamily. Most of them have the motor domain at the N-terminus of the heavy chain and walk toward the plus end of the microtubule. A particularly interesting family has the motor domain at the C-terminus and walks in the opposite direction, toward the minus end of the microtubule.
  -
- Most kinesins carry a binding site in the tail for either a membrane-enclosed organelle or another microtubule. Many of the kinesin superfamily members have specific roles in mitotic and meiotic spindle formation and chromosomes separation during cell division.



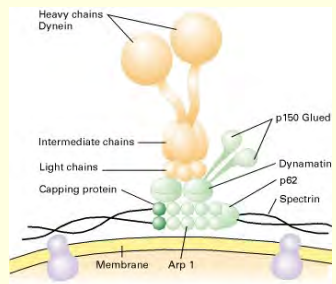
Adopted from Lodish H et al. Molecular Cell Biology. 4th edition. New York: W. H. Freeman; 2000., original source: M. Thormahlen et al., 1998, *J. Struc. Biol.* **122**:30–41.



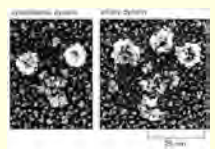
Adopted from Alberts 2008

There Are Two Types of Microtubule Motor Proteins: Kinesins and Dyneins

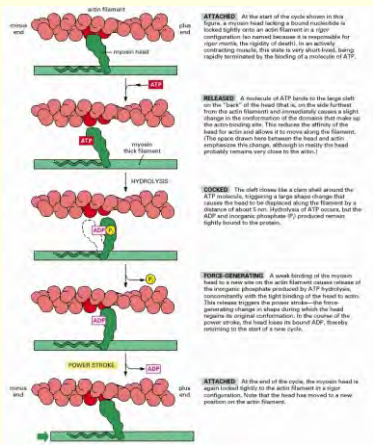
- The ***dyneins*** are a family of **inward**-directed microtubule motors, but they are unrelated to the kinesin superfamily. They are composed of two or three heavy chains (that include the motor domain) and a large and variable number of associated light chains. The dynein family has two major branches :
  - The most ancient branch contains the ***cytoplasmic*** *dyneins,* which are typically heavy-chain homodimers, with two large motor domains as heads. Cytoplasmic dyneins are probably found in all eucaryotic cells, and they are important for vesicle trafficking, as well as for localization of the Golgi apparatus near the center of the cell.
  - ***Axonemal*** *dyneins,* the other large branch, include heterodimers and heterotrimers, with two or three motor-domain heads, respectively. They are highly specialized for the rapid and efficient sliding movements of microtubules that drive the beating of cilia and flagella. A third, minor, branch shares greater sequence similarity with cytoplasmic than with axonemal dyneins but seems to be involved in the beating of cilia.
- ***Dyneins*** are the largest of the known molecular motors, and they are also among the fastest: axonemal dyneins can move microtubules in a test tube at the remarkable rate of **14 µm/sec**. In comparison, the fastest ***kinesins*** can move their microtubules at about **2–3 µm/sec**.

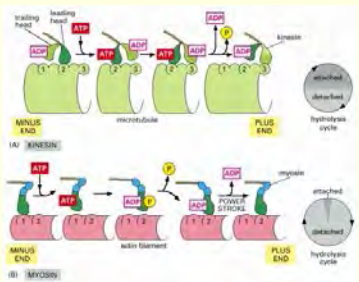


Adopted from Lodish H et al. Molecular Cell Biology. 4th edition. New York: W. H. Freeman; 2000., original source: N. Hirokawa, 1998, *Science* **279:**519.



Motor Proteins Generate Force by Coupling ATP Hydrolysis to Conformational Changes

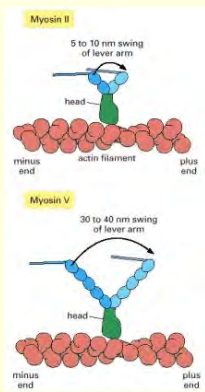




Adopted from Alberts 2008

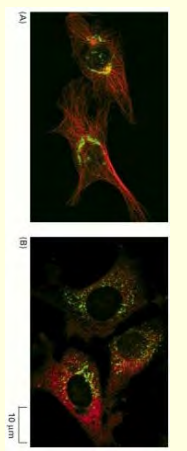
Motor Protein Kinetics Are Adapted to Cell Functions

- each motor protein class, movement speeds vary widely
  - from about 0.2 to 60 µm/sec - myosins,
  - from about 0.02 to 2 µm/sec - kinesins.
  - Up to 14 µm/sec - dyneins
- The number of steps that an individual motor molecule can take in a given time, and thereby the velocity, can be increased by either increasing the motor protein's intrinsic ATPase rate or decreasing the proportion of cycle time spent bound to the filament track.
- The size of each step can be changed by either changing the length of the lever arm (for example, the lever arm of myosin V is about three times longer than the lever arm of myosin II) or the angle through which the helix swings
- The behavior of each motor protein, whose function is determined by the identity of the cargo attached through its tail-domain, has been fine-tuned during evolution for speed and effectiveness according to the specific needs of the cell



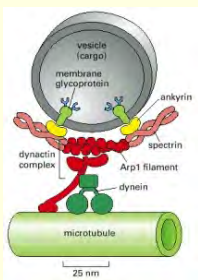
Motor Proteins Mediate the Intracellular Transport of Membrane-enclosed Organelles

- A major function of cytoskeletal motors in interphase cells is the transport and positioning of membrane-enclosed organelles.
- Kinesin was originally identified as the protein responsible for fast axonal transport, the rapid movement of mitochondria, secretory vesicle precursors, and various synapse components down the microtubule highways of the axon to the distant nerve terminals. Although organelles in most cells need not cover such long distances, their polarized transport is equally necessary.
  - A typical microtubule array in an interphase cell is oriented with the minus end near the center of the cell at the centrosome, and the plus end extending to the cell periphery.
  - centripetal movements of organelles toward the cell center require the action of minus-end-directed motor proteins such as cytoplasmic dynein,
  - centrifugal movements toward the periphery require plus-end-directed motors such as kinesins.



Motor Proteins Mediate the Intracellular Transport of Membrane-enclosed Organelles

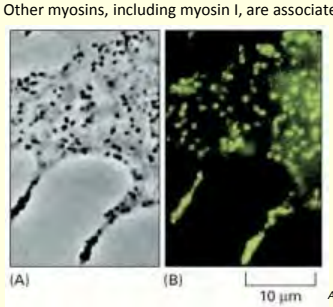
- The role of microtubules and microtubule motors in the behavior of intracellular membranes is best illustrated by the part they play in organizing the ER and the Golgi apparatus. The network of ER membrane tubules aligns with microtubules and extends almost to the edge of the cell, whereas the Golgi apparatus is located near the centrosome.
  - *In vitro*, kinesins can tether ER-derived membranes to preformed microtubule tracks, and walk toward the microtubule plus end, dragging the ER membranes out into tubular protrusions and forming a membranous web very much like the ER in cells. Likewise, the outward movement of ER tubules toward the cell periphery is associated with microtubule growth in living cells.
  - Conversely, dyneins are required for positioning the Golgi apparatus near the cell center, moving Golgi vesicles along microtubule tracks toward minus ends at the centrosome.
- The different tails and their associated light chains on specific motor protein allow the motors to attach to their appropriate organelle cargo. For example, there is evidence for membrane-associated motor receptors, sorted to specific membrane-enclosed compartments, that interact directly or indirectly with the tails of the appropriate kinesin family members.
  - One of these receptors seems to be the amyloid precursor protein, APP, which binds directly to a light chain on the tail of kinesin-I and is proposed to be a transmembrane motor protein receptor molecule in nerve-cell axons. It is the abnormal processing of this protein that gives rise to Alzheimer's disease.



Adopted from Alberts 2008

Motor Proteins Mediate the Intracellular Transport of Membrane-enclosed Organelles

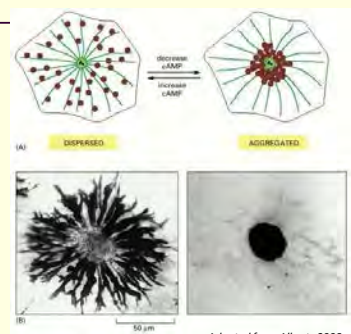
- Motor protein also have a significant role in organelle transport along actin filaments.
- In mice (and humans), membrane-enclosed pigment granules, called *melanosomes,* are synthesized in cells called *melanocytes* beneath the skin surface. These melanosomes move out to the ends of dendritic processes in the *melanocytes*, from where they are delivered to the overlying keratinocytes that form the skin and fur. Myosin V is associated with the surface of melanosomes
- Other myosins, including myosin I, are associated with endosomes and a variety of other organelles.



Adopted from Alberts 2008

Motor Protein Function Can Be Regulated

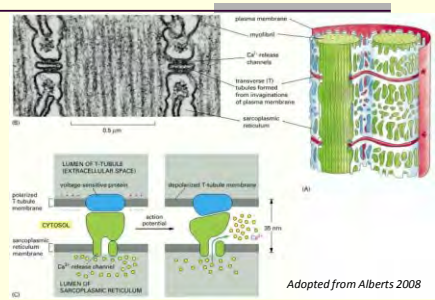
- The cell can regulate the activity of motor protein , allowing it to change either the positioning of its membrane-enclosed organelles or its whole-cell movements.
  - in response to neuronal or hormonal stimulation
- the movement is controlled by a complex balance of competing signals that regulate both motor protein attachment and activity usually via phosphorylation and dephosphorylation



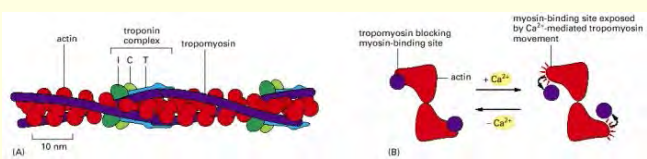
Adopted from Alberts 2008

Motor Protein Function Can Be Regulated

- The cell can regulate the activity of motor protein, allowing it to change either the positioning of its membrane-enclosed organelles or its whole-cell movements.
  - in response to neuronal or hormonal stimulation
- The movement is controlled by a complex balance of competing signals that regulate both motor protein attachment and activity usually via phosphorylation and dephosphorylation



Adopted from Alberts 2008



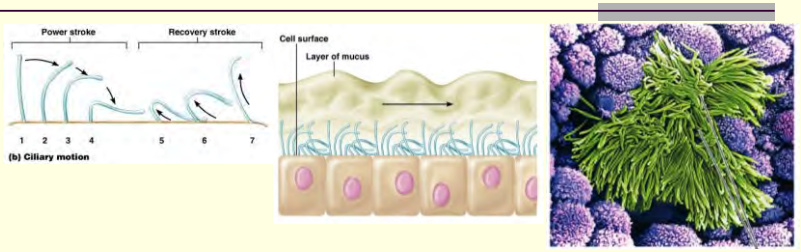
Adopted from Alberts 2008

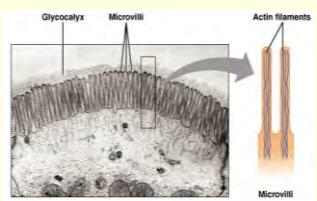
Cilia and Flagella Are Motile Structures Built from Microtubules and Dyneins

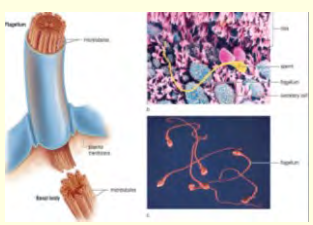
- Just as myofibrils are highly specialized and efficient motility machines built from actin and myosin filaments, ***cilia and flagella*** are highly specialized and efficient motility structures built from microtubules and dynein. Both cilia and flagella are hair-like cellular appendages that have a bundle of microtubules at their core.
- ***Flagella*** are found on sperm and many protozoa. By their undulating motion, they enable the cells to which they are attached to swim through liquid media.
- ***Cilia*** tend to be shorter than flagella and are organized in a similar fashion, but they beat with a whip-like motion that resembles the breast stroke in swimming. The cycles of adjacent cilia are almost but not quite in synchrony, creating the wave-like patterns that can be seen in fields of beating cilia under the microscope.
  - Ciliary beating can either propel single cells through a fluid (as in the swimming of the protozoan *Paramecium*) or can move fluid over the surface of a group of cells in a tissue. In the human body, huge numbers of cilia (10^9/cm^2 or more) line our respiratory tract, sweeping layers of mucus, trapped particles of dust, and bacteria up to the mouth where they are swallowed and ultimately eliminated. Likewise, cilia along the oviduct help to sweep eggs toward the uterus.

Adopted from Alberts 2008

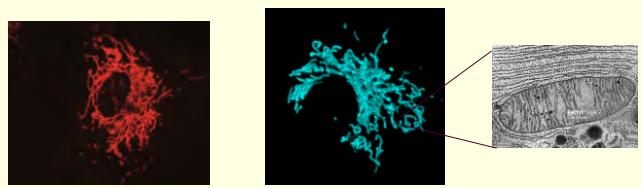
Cilia & Flagella







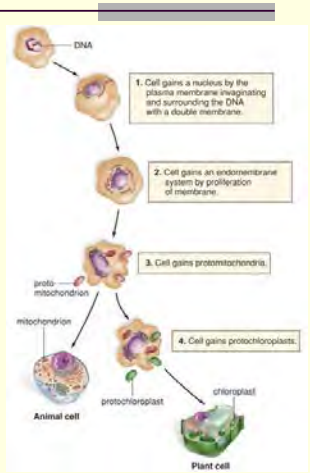
Adopted from Fox 2009



Mitochondria

Mitochondria and Chloroplasts Probably Both Evolved from Endosymbiotic Bacteria

- The prokaryotic character of the organelle genetic systems, especially striking in chloroplasts, suggests that mitochondria and chloroplasts evolved from bacteria that were endocytosed more than **1 billion years ago**.
- According to *endosymbiont hypothesis*, eukaryotic cells started out as anaerobic organisms without mitochondria or chloroplasts and then established a stable endosymbiotic relation with a bacterium, whose oxidative phosphorylation system they subverted for their own use.
- The endocytic event that led to the development of mitochondria is presumed to have occurred when oxygen reached substantial concentration in the atmosphere, about 1.5×10^9 years ago, before animals and plants separated.
- Plant and algal chloroplasts seem to have been derived later from an endocytic event involving an oxygen-producing photosynthetic bacterium.
  - To explain the different pigments and properties of the chloroplasts, it is usually assumed that at least three independent endosymbiotic events occurred.



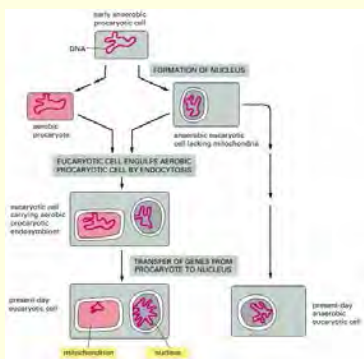
Adopted from Silverthorn 2010

Endosymbiont Hypothesis

- ***Endosymbiont hypothesis***: originally proposed in 1883 by Andreas Schimper, but drawn-out by Lynn Margulis in the 1980s.
- Mitochondrial ribosomal RNA genes and other genes show that the original organism was in the alpha-proteobacterial family (similar to nitrogen-fixing bacteria)
- **Evidence**:
  - mitochondria have their *own circular DNA*
  - the *inner membrane is more similar to prokaryotic membranes* than to eukaryotic. By the hypothesis, the inner membrane was the original prokaryotic membrane and the outer membrane was from the primitive eukaryote that swallowed it.
  - mitochondria make their *own ribosomes, which are of the prokaryotic 70S type*, not the eukaryotic 80S type.
  - *mitochondria are sensitive to many bacterial inhibitors* that don't affect the rest of the eukaryotic cell, such as streptomycin, chloramphenicol, rifampicin.
  - *mitochondrial protein synthesis starts with N-formyl methionine, as in the bacteria* but unlike eukaryotes.
- Most of the original bacterial genes have migrated into the nucleus.
- Eukaryotes that lack mitochondria generally have some mitochondrial genes in their nucleus, evidence that their ancestors had mitochondria that were lost during evolution.

Mitochondria and Chloroplasts Probably Both Evolved from Endosymbiotic Bacteria

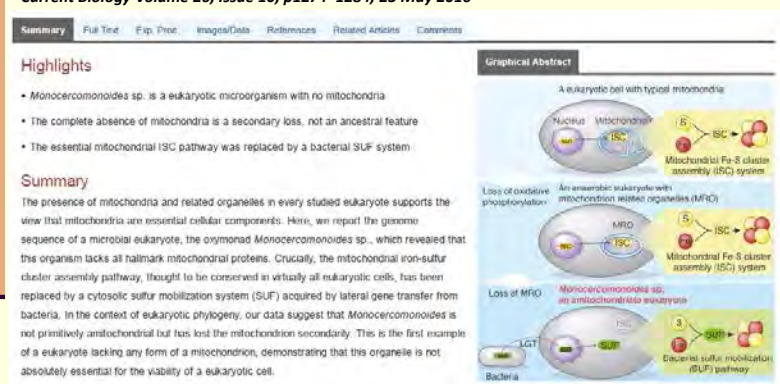
- **What type of bacterium gave rise to the mitochondrion?** From sequence comparisons, it seems that mitochondria are descendants of a particular type of ***purple photosynthetic bacterium*** that had previously lost its ability to perform photosynthesis and was left with only a respiratory chain.
  - It is not certain whether all mitochondria have originated from the same endosymbiotic event, however.
- *Microsporidia* and *Giardia* are two present-day anaerobic single-celled eukaryotes (protozoans) without mitochondria. Because they have an rRNA sequence that suggests a great deal of evolutionary distance from all other known eukaryotes.
- Most of the genes encoding present-day mitochondrial and chloroplast proteins are in the cell nucleus. Thus, an extensive transfer of genes from organelle to nuclear DNA must have occurred during eukaryote evolution.
- Gene transfer seems to have been a gradual process. When mitochondrial genomes encoding different numbers of proteins are compared, a pattern of sequential reduction of encoded mitochondrial functions emerges .



Adopted from Alberts 2008

A Eukaryote without a Mitochondrial Organelle

Current Biology Volume 26, Issue 10, p1274–1284, 23 May 2016

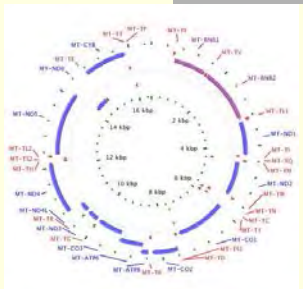


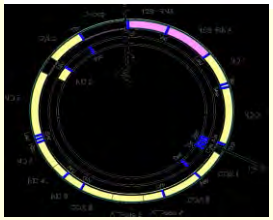
Monocercomonoides sp.

Mitochondrial genome

- ***Mitochondrial DNA*** or ***mtDNA***. In humans, mitochondrial DNA spans about 16,500 (16,5 kb) DNA building blocks (base pairs), representing a small fraction of the total DNA in cells.
- Mitochondrial DNA contains ***37 genes***, all of which are essential for normal mitochondrial function.
- Thirteen of these genes encode the enzymes involved in oxidative phosphorylation. Oxidative phosphorylation is a process that uses oxygen and simple sugars to create adenosine triphosphate (ATP), the cell's main energy source.
- The remaining genes encode transfer RNA (tRNA) and ribosomal RNA (rRNA), which are chemical cousins of DNA. The RNAs help assemble protein building blocks (amino acids) into functioning proteins.
- Mitochondrial genes (37 genes) are among the estimated 20,000 to 25,000 total genes in the human genome.

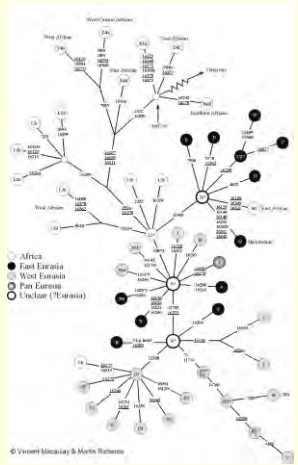
http://ghr.nlm.nih.gov/
A service of the **U.S. National Library of Medicine®**





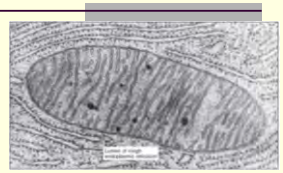
Mitochondrial Genetics

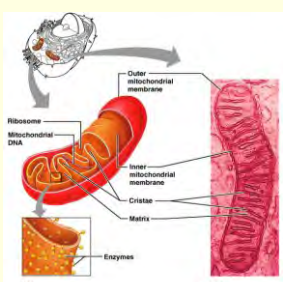
- ***Maternal inheritance***: Mitochondrial genome is inherited through the mother only (ovum). It allows to trace female lineage back in time.
- A few sperm mitochondria enter the egg, but they are degraded and lost.
- ***Mutation rate*** in mtDNA is very high: 10 times the nuclear rate. mtDNA is associated with the inner membrane, the site of oxidative phosphorylation. Large amounts of "reactive oxygen species" (peroxide and superoxide) are present, and they are quite mutagenic. Part of the effects of aging have been attributed to the gradual loss of mitochondria due to accumulated mutations in individual cells.



The general organization of a mitochondrion

- ***Complex organelles*** – contain their own DNA and RNA and are able to reproduce themselves
- **Shape** – Rod or oval shape surrounded by two membranes
- **Function** – Major site of ATP production, O_2 consumption and CO_2 formation, Ca^{2+} homeostasis
- Each mitochondrion is bounded by two highly specialized membranes, which have very different functions. Together they create two separate mitochondrial compartments: the internal **matrix** and a much narrower intermembrane space.
- The ***outer membrane*** contains many copies of a transport protein called *porin*, which forms large aqueous channels through the lipid bilayer. This membrane thus resembles a sieve that is permeable to all molecules of 5000 daltons or less, including small proteins. Such molecules can enter the intermembrane space, but most of them cannot pass the impermeable inner membrane.
  - the intermembrane space is chemically equivalent to the cytosol with respect to the small molecules it contains,
  - the matrix contains a highly selected set of these molecules.
- ***The inner membrane*** is highly specialized. Its lipid bilayer contains a high proportion of the "double" phospholipid ***cardiolipin***, which has four fatty acids rather than two and may help to make the membrane **especially impermeable to ions**. This membrane also contains a variety of **transport proteins** that make it selectively permeable to those small molecules that are metabolized or required by the many mitochondrial enzymes concentrated in the matrix.
- The inner membrane is usually highly convoluted, forming a series of invaginations, known as ***cristae***, that project into the ***matrix*** – increase of area for ATP production

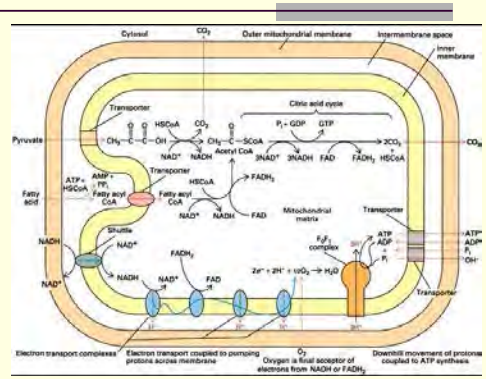




Adopted from Fox 2009

The inner membrane and matrix

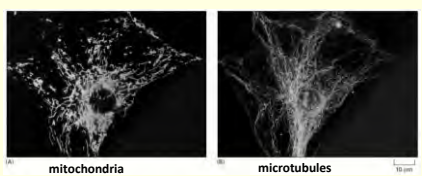
- The matrix enzymes include those that metabolize pyruvate and fatty acids to produce acetyl CoA and those that oxidize acetyl CoA in the ***citric acid cycle***.
- The principal end-products of this oxidation are CO_2, which is released from the cell as waste, and NADH, which is the main source of electrons for transport along the ***ETC*** —the name given to the electron-transport chain in mitochondria.
  - The enzymes of the respiratory chain are embedded in the inner mitochondrial membrane, and they are essential to the process of *oxidative phosphorylation,* which generates most of the animal cell's ATP.



- **Function**:
  - Major site of ATP production,
  - O_2 consumption and CO_2 formation,
  - Ca^{2+} homeostasis

Mitochondria localization

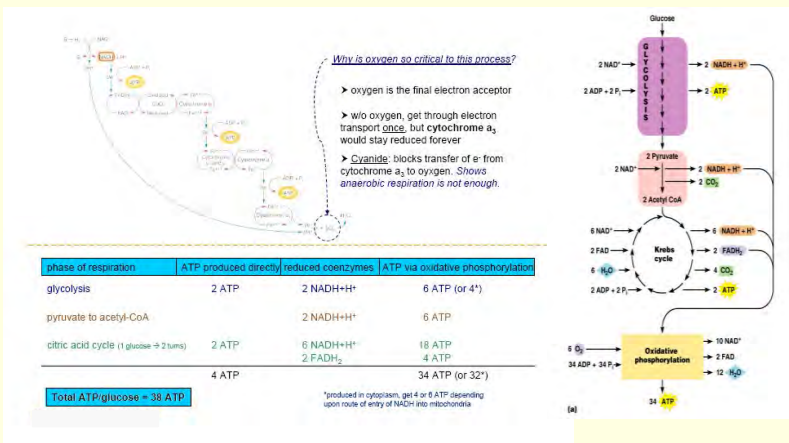
- mitochondria tend to be aligned along microtubules



- mitochondria near sites of high ATP utilization

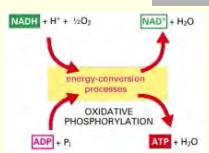
Adopted from Fox 2009

Summary of Glucose Oxidation

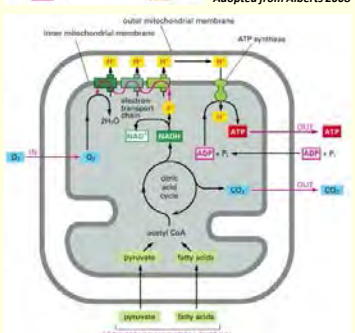


A summary of energy-generating metabolism in mitochondria

- In the process of oxidative phosphorylation, the inner mitochondrial membrane serves as a device that changes one form of chemical bond energy to another, converting a major part of the energy of NADH (and $FADH_2$) oxidation into phosphate-bond energy in ATP.
- Pyruvate and fatty acids enter the mitochondrion *(bottom)* and are broken down to acetyl CoA. The acetyl CoA is then metabolized by the citric acid cycle, which reduces NAD^+ to NADH (and FAD to $FADH_2$, not shown). In the process of oxidative phosphorylation, high-energy electrons from NADH (and $FADH_2$) are then passed along the electron-transport chain in the inner membrane to oxygen (O_2). This electron transport generates a proton gradient across the inner membrane, which is used to drive the production of ATP by ATP synthase
- The NADH generated by glycolysis in the cytosol also passes electrons to the respiratory chain (not shown). Since NADH cannot pass across the inner mitochondrial membrane, the electron transfer from cytosolic NADH must be accomplished indirectly by means of one of several "shuttle" systems that transport another reduced compound into the mitochondrion; after being oxidized, this compound is returned to the cytosol, where it is reduced by NADH again.

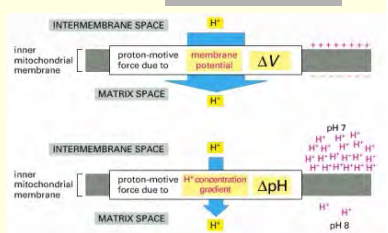


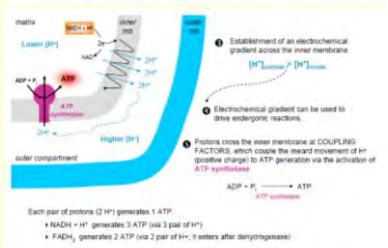
Adopted from Alberts 2008



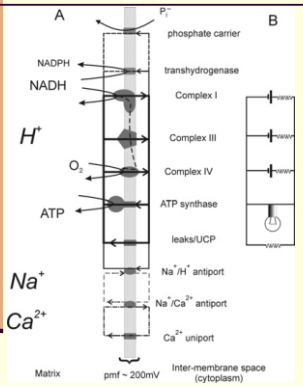
As Electrons Move Along the Respiratory Chain, Energy Is Stored as an Electrochemical Proton Gradient Across the Inner Membrane – Chemiosmotic Coupling

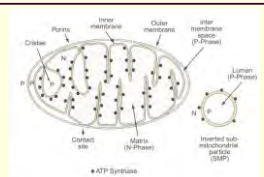
- Oxidative phosphorylation is made possible by the close association of the electron carriers with protein molecules. The proteins guide the electrons along the respiratory chain so that the electrons move sequentially from one enzyme complex to another—with no short circuits. Most importantly, the transfer of electrons is coupled to oriented H^+ uptake and release, as well as to allosteric changes in energy-converting protein pumps. The net result is the pumping of H^+ across the inner membrane—from the matrix to the intermembrane space, driven by the energetically favorable flow of electrons. This movement of H^+ has ***two major consequences***:
  - It generates a pH gradient across the inner mitochondrial membrane, with the pH higher in the matrix than in the cytosol, where the pH is generally close to 7. (Since small molecules equilibrate freely across the outer membrane of the mitochondrion, the pH in the intermembrane space is the same as in the cytosol.)
  - It generates a voltage gradient *(membrane potential Ψm)* across the inner mitochondrial membrane, with the inside negative and the outside positive (as a result of the net outflow of positive ions).
- The pH gradient (ΔpH) drives H^+ back into the matrix and OH^- out of the matrix, thereby reinforcing the effect of the membrane potential (Δ*V*), which acts to attract any positive ion into the matrix and to push any negative ion out. Together, the ΔpH and the Δ*V* are said to constitute an ***electrochemical proton gradient***. The electrochemical proton gradient exerts a, ***proton-motive force (Δ p)***, which can be measured in units of millivolts (mV).
  - **pmf = *Δ p = ψm − 61.5 log10 pH, where Ψm = 61.5 log10 [C⁺in]/ [C⁺out]***
- Since each ΔpH of 1 pH unit has an effect equivalent to a membrane potential of about 60 mV, the total proton-motive force equals Δ*V* - 60(ΔpH). In a typical cell, the proton-motive force across the inner membrane of a respiring mitochondrion is about 200 mV and is made up of a membrane potential of about 140 mV and a pH gradient of about -1 pH unit.

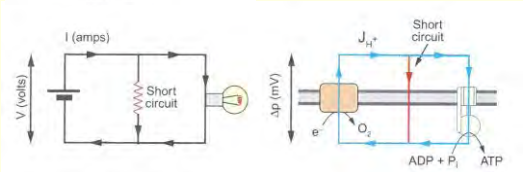




Circuits of protonmotive force







pmf = $\Delta p = \psi_m - 61.5 \log 10\ pH$,

where $\Psi_m = 61.5 \log 10\ [C^+_{in}]/[C^+_{out}]$

Analogy to electrical circuit obeying the Ohm law I=V/R :

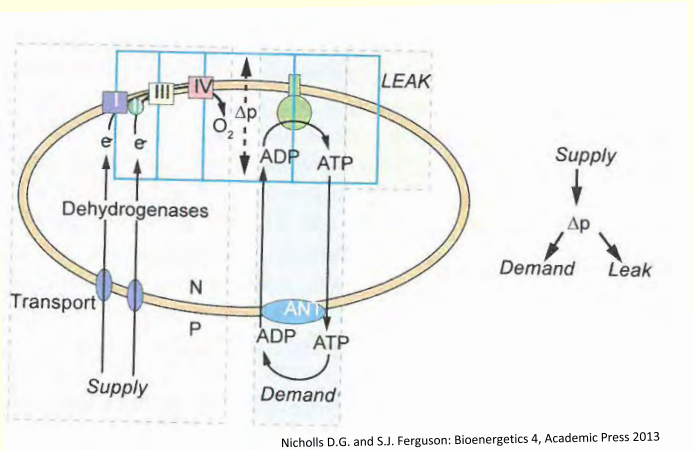
Open circuit = no respiration, zero current, potential is maximal

Closed circuit = respiration, current flows, potential decreases and ATP is synthesised

Short circuit = addition of protonofore, potential is low, respiration high

Nicholls D.G. and S.J. Ferguson: Bioenergetics 4, Academic Press 2013

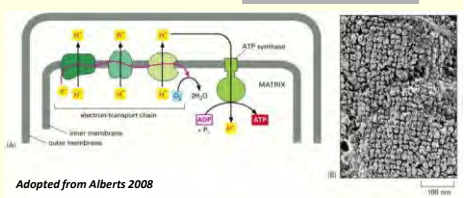
Modules for metabolic analysis



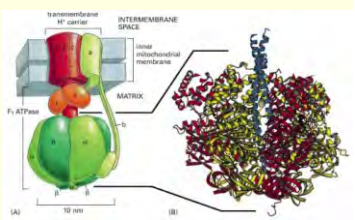
Nicholls D.G. and S.J. Ferguson: Bioenergetics 4, Academic Press 2013

The Proton Gradient Drives ATP Synthesis

- The electrochemical proton gradient across the inner mitochondrial membrane drives ATP synthesis in the critical process of oxidative phosphorylation.
  - The membrane-bound enzyme ***ATP synthase***. This enzyme creates a hydrophilic pathway across the inner mitochondrial membrane that allows protons to flow down their electrochemical gradient. As these ions thread their way through the ATP synthase, they are used to drive the energetically unfavorable reaction between ADP and P_i that makes ATP. **The ATP synthase is of ancient origin**; the same enzyme occurs in the mitochondria of animal cells, the chloroplasts of plants and algae, and in the plasma membrane of bacteria and archaea.
- The structure of ATP synthase
  - Also called the F_0F_1 ATPase



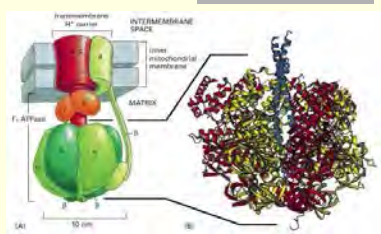
Adopted from Alberts 2008



Adopted from Alberts 2008 (*B, originally from J.P. Abrahams et al., Nature 370:621–628, 1994.*)

How the Proton Gradient Drives ATP Synthesis

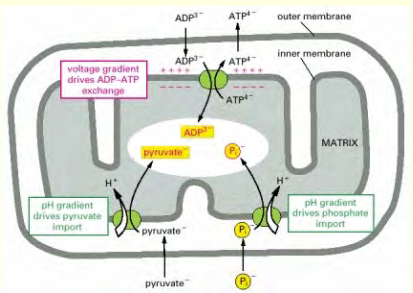
- Also called the F_0F_1 ATPase, it is a multisubunit protein with a mass of more than 500,000 daltons.
  - (**A**) The enzyme is composed of a ***head portion***, called the ***F_1 ATPase***, and a ***transmembrane H^+ carrier***, called ***F_0***. Both F_1 and F_0 are formed from multiple subunits, as indicated. A rotating stalk turns with a rotor formed by a ring of 10 to 14 c subunits in the membrane *(red).* The stator *(green)* is formed from transmembrane a subunits, tied to other subunits that create an elongated arm. This arm fixes the stator to a ring of 3α and 3β subunits that forms the head.
  - (**B**) The three-dimensional structure of the F_1 ATPase, determined by x-ray crystallography. This part of the ATP synthase derives its name from its ability to carry out the reverse of the ATP synthesis reaction—namely, the hydrolysis of ATP to ADP and P_i, when detached from the transmembrane portion.



- As protons pass through a narrow channel formed at the stator-rotor contact, their movement causes the rotor ring to spin. This spinning also turns a stalk attached to the rotor, which is thereby made to turn rapidly inside the lollipop head. As a result, the energy of proton flow down a gradient has been converted into the mechanical energy of two sets of proteins rubbing against each other: rotating stalk proteins pushing against a stationary ring of head proteins.
- Three of the six subunits in the head contain binding sites for ADP and inorganic phosphate. These are driven to form ATP as mechanical energy is converted into chemical bond energy through the repeated changes in protein conformation that the rotating stalk creates. In this way, ***the ATP synthase is able to produce more than 100 molecules of ATP per second. Three or four protons need to pass through this device to make each molecule of ATP.***

Some of the active transport processes driven by the electrochemical proton gradient across the inner mitochondrial membrane

- The synthesis of ATP is not the only process driven by the electrochemical proton gradient. In mitochondria, many charged small molecules, such as pyruvate, ADP, and P_i, are pumped into the matrix from the cytosol, while others, such as ATP, must be moved in the opposite direction. Carrier proteins that bind these molecules can couple their transport to the energetically favorable flow of H^+ into the mitochondrial matrix.
- **Pyruvate**, inorganic phosphate (P_i), and **ADP** are moved into the matrix, while ATP is pumped out. The charge on each of the transported molecules is indicated for comparison with the membrane potential, which is negative inside, as shown. The outer membrane is freely permeable to all of these compounds.
- Because of the carrier protein in the inner mitochondrial membrane that exchanges ATP for ADP, the ADP molecules produced by ATP hydrolysis in the cytosol rapidly enter mitochondria for recharging, while the ATP molecules formed in the mitochondrial matrix by oxidative phosphorylation are rapidly pumped into the cytosol, where they are needed. A typical ATP molecule in the human body shuttles out of a mitochondrion and back into it (as ADP) for recharging more than once per minute, keeping **the concentration of ATP in the cell about 10 times higher than that of ADP**.



Adopted from Alberts 2008

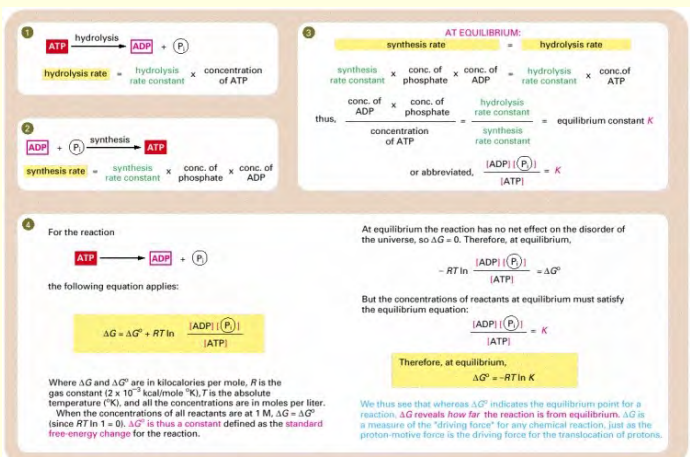
A Large Negative Value of ΔG for ATP Hydrolysis Makes ATP Useful to the Cell

- **ATP is the major "activated carrier molecule" in cells. The large, favorable free-energy change (large negative ΔG) for its hydrolysis is used, through *coupled reactions*, to drive other chemical reactions that would otherwise not occur. The ATP hydrolysis reaction produces two products, ADP and inorganic phosphate (P_i); it is therefore of the type A → B + C, where,**

$$\Delta G = \Delta G^{o} + RT\ ln\frac{[B][C]}{[A]}$$

- **When ATP is hydrolyzed to ADP and P_i under the conditions that normally exist in a cell, the free-energy change is roughly -11 to -13 kcal/mole. This extremely favorable ΔG depends on having a high concentration of ATP in the cell compared with the concentration of ADP and P_i. When ATP, ADP, and P_i are all present at the same concentration of 1 mole/liter (so-called standard conditions), the ΔG for ATP hydrolysis is the standard free-energy change (ΔG°), which is only -7.3 kcal/mole. At much lower concentrations of ATP relative to ADP and P_i, ΔG becomes zero. At this point, the rate at which ADP and P_i will join to form ATP will be equal to the rate at which ATP hydrolyzes to form ADP and P_i. In other words, when $\Delta G = 0$, the reaction is at *equilibrium***
- **It is ΔG, not ΔG°, that indicates how far a reaction is from equilibrium and determines whether it can be used to drive other reactions. Because the efficient conversion of ADP to ATP in mitochondria maintains such a high concentration of ATP relative to ADP and P_i, the ATP-hydrolysis reaction in cells is kept very far from equilibrium and ΔG is correspondingly very negative. Without this large disequilibrium, ATP hydrolysis could not be used to direct the reactions of the cell; for example, many biosynthetic reactions would run backward rather than forward at low ATP concentrations.**

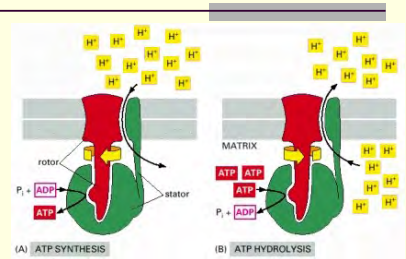
The basic relationship between free-energy changes and equilibrium in the ATP hydrolysis reaction



Adopted from Alberts 2008

The ATP synthase is a reversible coupling device that can convert the energy of the electrochemical proton gradient into chemical-bond energy, or vice versa

- The ATP synthase can either (A) **synthesize ATP** by harnessing the proton-motive force or (B) **pump protons** against their electrochemical gradient by hydrolyzing ATP.
- The direction of operation at any given instant depends on the net free-energy change (Δ*G*) for the coupled processes of H^+ translocation across the membrane and the synthesis of ATP from ADP and P_i.
- Measurement of the torque that the ATP synthase can produce when hydrolyzing ATP reveals that the synthase can pump 60 times more strongly than a diesel-engine of equal weight.
- The free-energy change (Δ*G*) for ATP hydrolysis depends on the concentrations of the three reactants ATP, ADP, and P_i;
- The Δ*G* for ATP synthesis is the negative.



Adopted from Alberts 2008

- The Δ*G* for proton translocation across the membrane is proportional to the proton-motive force.
- The conversion factor between them is the faraday. Thus, ΔG_{H^+} = -0.023 (proton-motive force), where ΔG_{H^+} is in kcal/mole and the proton-motive force is in mV. For an electrochemical proton gradient (proton-motive force) of 200 mV,
- **ΔG_{H^+} = -4.6 kcal/mole**

Summary of mitochondrion functions

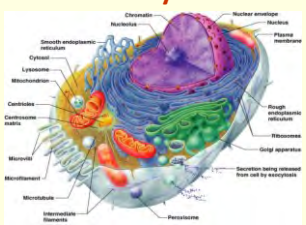
- The mitochondrion performs most cellular oxidations and produces the bulk of the animal cell's ATP. The mitochondrial matrix contains a large variety of enzymes, including those that convert pyruvate and fatty acids to acetyl CoA and those that oxidize this acetyl CoA to CO_2through the citric acid cycle. Large amounts of NADH (and $FADH_2$) are produced by these oxidation reactions.
- The energy available from combining molecular oxygen with the reactive electrons carried by NADH and $FADH_2$ is harnessed by an electron-transport chain in the inner mitochondrial membrane called the respiratory chain. The respiratory chain pumps H^+ out of the matrix to create a transmembrane electrochemical proton (H^+) gradient, which includes contributions from both a membrane potential and a pH difference. The large amount of free energy released when H^+ flows back into the matrix (across the inner membrane) provides the basis for ATP production in the matrix by a remarkable protein machine—the ATP synthase. The transmembrane electrochemical gradient is also used to drive the active transport of selected metabolites across the mitochondrial inner membrane, including an efficient ATP-ADP exchange between the mitochondrion and the cytosol that keeps the cell's ATP pool highly charged. The resulting high ratio of ATP to its hydrolysis products makes the free-energy change for ATP hydrolysis extremely favorable, allowing this hydrolysis reaction to drive a large number of the cell's energy-requiring processes.

Mitochondria and Cell Death

Eukaryotic cellular signaling

- Compartmentalization is one of the key features of eukaryotic cellular signaling. The requirement for spatial and temporal limitation of amplifying second messengers stems from their pleiotropic nature. One single messenger, like Ca^{2+}, can control cell proliferation or death: the outcome is determined by the strength, the localization, the duration and the pattern of the signal.
- Organelles are key participants in the amplification of signaling cascades, either because they regulate the production or the release of crucial second messengers, or because they are strategically located in the cytoplasm at the sites of signal propagation.
- Organelle localization is often crucial to properly modulate cellular functions and signaling cascades. For example, the distribution of organelles in axons is crucial for their function and is deregulated in several diseases.

Eukaryotic Cell



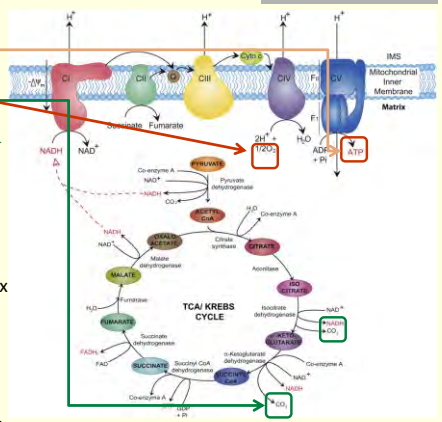
Adopted from Fox 2009

Mitochondria Function

- **Functions**
  - Major site of ATP production, O_2 consumption and CO_2 formation,
  - Ca^{2+} homeostasis
  - Cell survival or death

Major site of ATP production, O_2 consumption and CO_2 formation

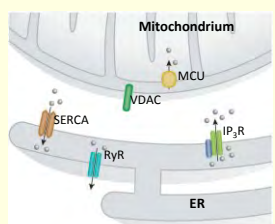
- Site of :
  - ATP production
  - O_2 consumption
  - CO_2 production
- *Bioenergetics of the electron transport chain and the TCA/Kerbs cycle.*
- Pyruvate is converted to high-energy molecules LIKE NADH, GTP and FADH2 through catalyzation by TCA/Kerbs cycle enzymes.
- NADH generated is shuttled to complex I and is converted to NAD^+ driving oxidative phosphorylation. Transfer of electrons through the chain maintains the membrane potential via proton pumping into the IMS.
- In the final step ADP is phosphorylated to form ATP via complex V (ATP synthase).

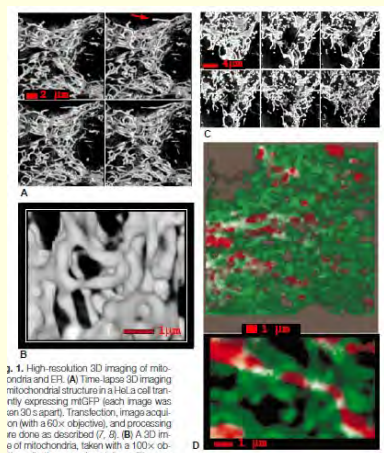


L.D. Osellame et al. / Best Practice & Research Clinical Endocrinology & Metabolism 26 (2012) 711–723

Mitochondria and Ca^{2+} homeostasis

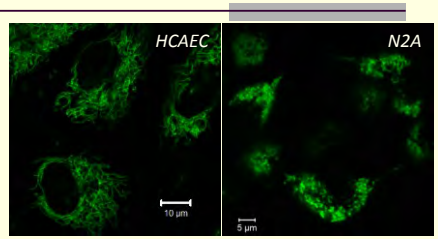
- The specific ER region that interacts with mitochondria has been christened mitochondria-associated membrane (**MAM**) by Jean Vance when she identified for the first time an important function for the intimate relationship between the two compartments in the exchange of phospholipids (Vance, 1990).
- The specific ER region that interacts with mitochondria - mitochondria-associated membrane (MAM)
  - Ca2+ homeostasis - a 'quasi-synaptic' mechanism transmission of Ca^{2+} between the two organelles (Rosario Rizzuto *et al. Science* **280**, 1763 (1998)
  - Fission sites (Bereiter-Hahn J & Voth M. Microscopy Research and Techniques 1994; 27: 198–219)

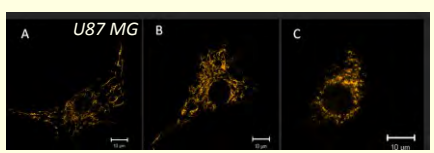




Mitochondria: A Dynamic Organelle

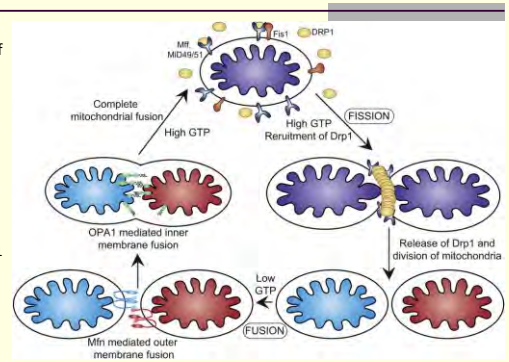
- The dynamic nature of mitochondria allows the adjustment of mitochondrial morphologies to specific cellular processes.
- Mitochondrial shape in living cells is very heterogeneous and can range from small spheres to interconnected tubules
- Mitochondrial architecture is not random, rather the opposing processes of fission and fusion specifically determine mitochondrial shape.
- The specific control of mitochondrial morphology has a significant impact on mitochondrial function and homeostasis.
- Mitochondrial fusion was suggested as a route for the rapid exchange of metabolites, mitochondrial DNA (mtDNA) and membrane components,
- Fission is thought to facilitate the segregation of mtDNA and isolation of mitochondria from the network to allow their degradation.
- Thus, mitochondrial fission and fusion influences nearly all aspects of mitochondrial function, including respiration, calcium buffering and apoptosis.





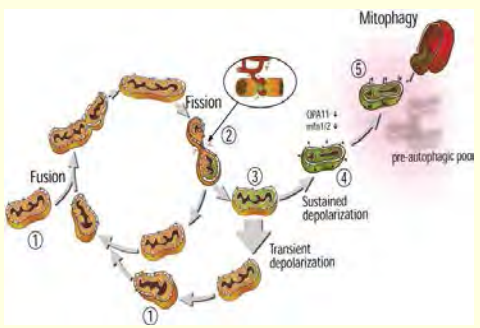
Mitochondrial dynamics

- Steady state mitochondrial morphology requires a balance of fission and fusion events.
- On the molecular level, mitochondrial morphology is controlled through a family of dynamin-related proteins.
- Organelle division is mediated by ***Drp1*** which forms high molecular weight oligomers on the mitochondrial surface. Once Drp1 is released fission is complete.
- Mitochondrial fusion is a two-step process that requires outer and inner membrane fusion.
- Outer membrane fusion is facilitated by ***mitofusin*** tethering of adjacent membranes.
- In high GTP environments, OPA1 isoforms allow inner membrane fusion.



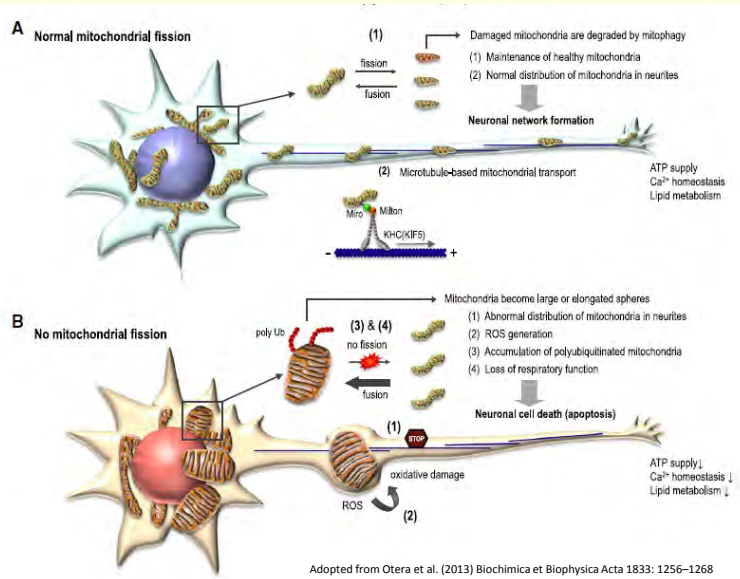
L.D. Osellame et al. / Best Practice & Research Clinical Endocrinology & Metabolism 26 (2012) 711–723
Rambold & Lippincott-Swarz 2011, Cell Cycle 10 (23): 4032-4038

Mitochondrial dynamics – quality control



L. Stiles, O.S. Shirihai / Best Practice & Research Clinical Endocrinology & Metabolism 26 (2012) 725–738
Rambold & Lippincott-Swarz 2011, Cell Cycle 10 (23): 4032-4038

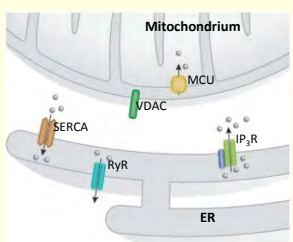
- The dynamic nature of mitochondria is also essential for mitochondrial quality control. Healthy mitochondria go through continuous fission and fusion cycles, which are, in general, timely coupled. In this process, after fusion takes place, it is rapidly followed by a mitochondrial fission event. Mitochondria then spend the vast amount of time in a post-fission state, which they only leave by re-fusing into the mitochondrial network. As mitochondrial fusion is dependent on membrane potential ($\Delta\psi_m$), mitochondrial depolarization will retain mitochondria in a post-fissioned state. The continuous failure of damaged mitochondria to fuse back into the mitochondrial network eventually leads to mitochondrial elimination (autophagy).



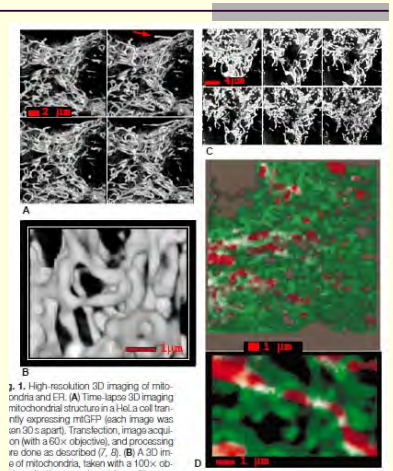
Adopted from Otera et al. (2013) Biochimica et Biophysica Acta 1833: 1256–1268

Mitochondria: A Dynamic Organelle

- The specific ER region that interacts with mitochondria - mitochondria-associated membrane (MAM)
  - Ca2+ homeostasis
  - Fission sites



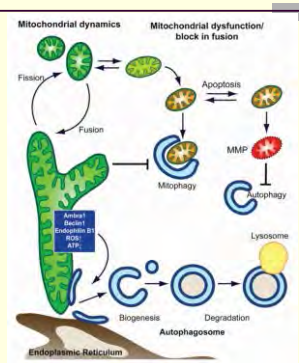
Rosario Rizzuto *et al. Science* **280**, 1763 (1998)
Bereiter-Hahn J & Voth M. Microscopy Research and Techniques 1994; 27: 198–219.

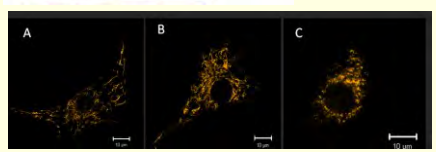


g. 1. High-resolution 3D imaging of mitochondria and ER. (**A**) Time-lapse 3D imaging mitochondrial structure in a HeLa cell transiently expressing mtGFP (each image was sec 30 s apart). Transfection, image acquisition (with a 60× objective), and processing re done as described (*7*, *8*). (**B**) A 3D image of mitochondria, taken with a 100× objective; all other experimental conditions as

Mitochondrial morphology, dynamics and apoptosis.

- **Rambold & Lippincott-Swarz 2011, Cell Cycle 10 (23): 4032-4038**
- **Cleland et al. 2011, Cell Death and Differentiation 18: 235–247**
- The mitochondrial reticulum can be rapidly remodeled by dynamic fission and fusion events in response to the physiological requirements of a cell.
- The mitochondrial network undergoes dramatic rearrangement upon induction of apoptosis, resulting in a fragmented mitochondrial phenotype and altered cristae junctions. Indicating that this process may be important for apoptosis, dominant-negative forms of Drp1 that antagonize mitochondrial division delay the release of cytochrome c and onset of cell death, although not as potently as some antiapoptotic Bcl-2 family members, such as Bcl-xL. Moreover, ectopic Mfn2, Opa1 and mutant forms of Opa1 can also confer protection against programmed cell death. Recently, several members of the Bcl-2 family, including both pro- and antiapoptotic proteins, have been shown to have a role in mitochondrial morphogenesis in healthy cells.



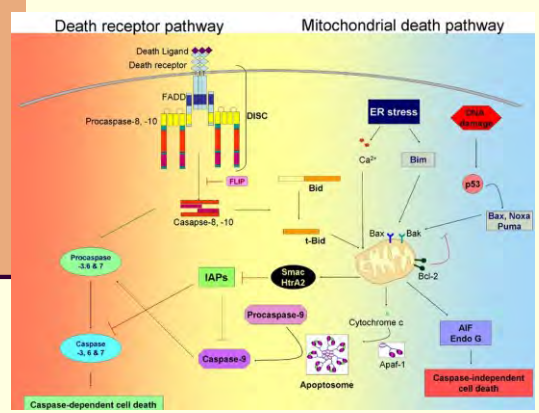


Apoptosis and mitochondria

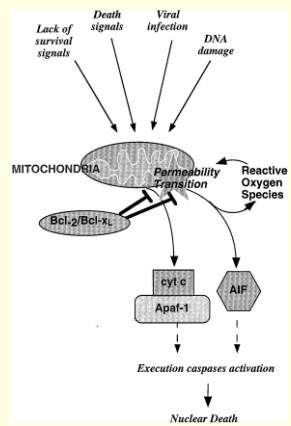
- Apoptosis is the typical form of programmed cell death (***PCD***) during development and adult cell turnover, however, it can also be triggered prematurely by a range of conditions occurring in diseases such as hypertension, atherosclerosis, and neurodegenerative diseases. Apoptosis plays a pivotal role in development, cancer, normal ageing and in neurological disorders such as Alzheimer's disease, amyotrophic lateral sclerosis and Parkinson's disease. A common feature of many neurological diseases is the degeneration of neuronal cells. It is widely accepted that neuronal loss in such diseases occurs by the inappropriate activation of apoptotic cell-death pathways. Apoptosis is induced via two main routes involving either the mitochondria *(the intrinsic pathway)* or the activation of death receptors (*the extrinsic pathway*). Both pathways converge to induce the activation of caspases the final executioners of cell death, although, it should be noted that caspase-independent forms of apoptosis have been reported (Leist and Jaattela 2001). Ultimately, apoptotic cells are ingested by neighbouring cells and phagocytes, preventing inflammation and tissue damage that might ensue upon cell lysis. The presence of the phospholipid phosphatidylserine (PS) on the outer leaflet of the plasma membrane acts as a signal for removal (Schlegel and Williamson 2001). Normally, cells maintain asymmetry of the inner and outer leaflets of the plasma membrane by actively translocating PS to the inner leaflet.

- Mitochondria play an important role in the regulation of cell death. They contain many pro-apoptotic proteins such as Apoptosis Inducing Factor (AIF), Smac/DIABLO and cytochrome C. These factors are released from the mitochondria following the formation of a pore in the mitochondrial membrane called the Permeability Transition pore, or PT pore. These pores are thought to form through the action of the pro-apoptotic members of the bcl-2 family of proteins, which in turn are activated by apoptotic signals such as cell stress, free radical damage or growth factor deprivation. Mitochondria also play an important role in amplifying the apoptotic signaling from the death receptors.

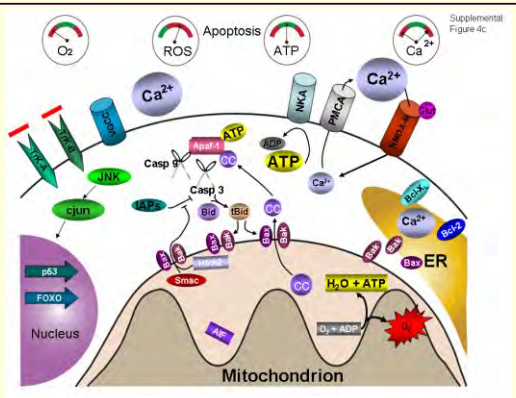
Apoptosis and mitochondria



Adopted from Gupta et al. (2009)



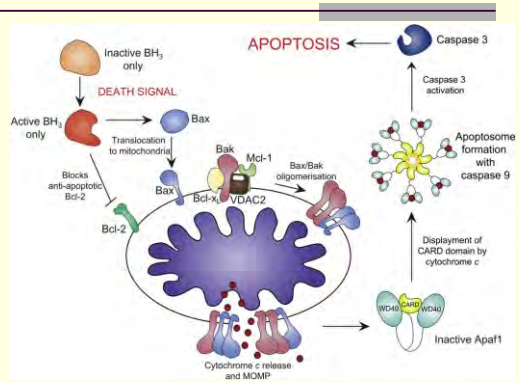
Involvement of mitochondria in intrinsic pathway of PCD.



- Cells undergoing apoptosis typically exhibit reduced oxygen consumption and increased ROS levels, but maintain ATP levels within a normal range. Apoptotic kinases such as JNK, and transcription factors such as cjun and p53 are activated resulting in the expression and mitochondrial translocation of pro-apoptotic Bcl2 proteins such as Bax and Bak resulting in the release of cytochrome C (CC). Upon entering the cytosol, CC induces the formation of the "apoptosome" in which it interacts with Apaf-1 and caspase-9. Activated caspase-9 then cleaves and activates caspase-3 which, in turn, cleaves various protein substrates that execute the cell death process. During apoptosis the integrity of cell membranes is maintained

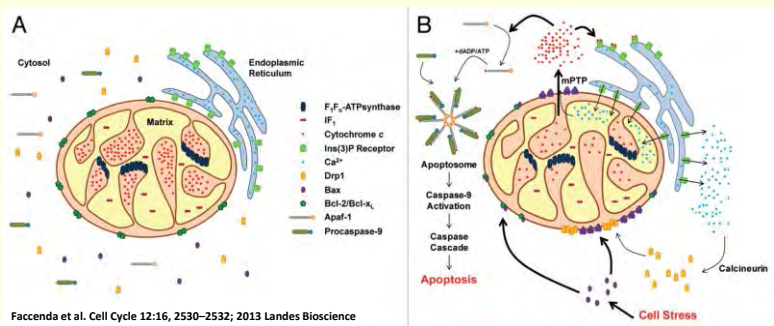
Apoptotic activation via the intrinsic pathway

- Apoptotic stimuli activates the BH3-only proteins, concurrently inactivating Bcl-2 and activating Bax translocation to mitochondria.
- Bak is held in check by Mcl-1, VDAC2 and Bcl-xL.
- Bax/Bak oligomerisation results in cytochrome c release and MOMP.
- Apaf-1 is activated by cytochrome c binding, displacing the CARD domain.
- The apoptosome forms with caspase-9, activating caspase-3 and triggering apoptosis.



L.D. Osellame et al. / Best Practice & Research Clinical Endocrinology & Metabolism 26 (2012) 711–723

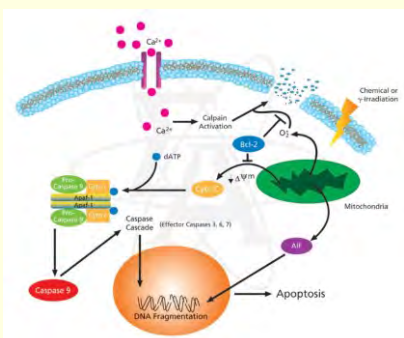
Regulation of apoptosis via mitochondria



Faccenda et al. Cell Cycle 12:16, 2530–2532; 2013 Landes Bioscience

Mitochondria and Ca^{2+} signaling in Apoptosis

- Increases in cytosolic Ca^{2+} levels due to activation of ion channel-linked receptors, such as that for the excitatory amino acid neurotransmitter glutamic acid, can induce ***permeability transition*** (PT) of the mitochondrial membrane. PT constitutes the first rate-limiting event of the common pathway of apoptosis. Upon PT, apoptogenic factors leak into the cytoplasm from the mitochondrial intermembrane space. Two such factors, ***cytochrome c*** and ***apoptosis inducing factor*** (**AIF**), begin a cascade of proteolytic activity that ultimately leads to nuclear damage (DNA fragmentation, DNA mutations) and cell death.
- Cytochrome c, a key protein in electron transport, appears to act by forming a multimeric complex with Apaf-1, a protease, which in turn activates procaspase 9, and begins a cascade of activation of downstream caspases. Smac/Diablo is released from the mitochondria and inhibits IAP (inhibitor of apoptosis) from interacting with caspase 9 leading to apoptosis.
- Anti-apoptotic Bcl-2 and Bcl-X can prevent pore formation and block the release of cytochrome c from the mitochondria and prevent activation of the caspase cascade and apoptosis. PT is also related to the mitochondrial generation of reactive oxygen species which plays a role in the degradation phase of apoptosis (i.e. plasma membrane alterations).



- **References**

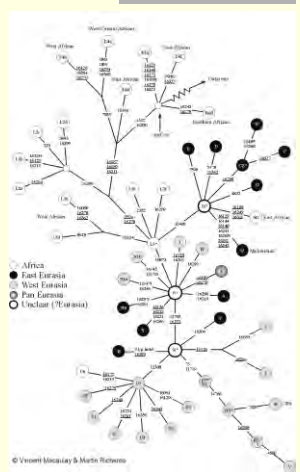
Budihardjo, I., et al., Biochemical pathways of caspase activation during apoptosis. Annu. Rev. Cell Dev. Biol., **15,** 269-290 (1999).
Susin, S.A., et al., Molecular characterization of mitochondrial apoptosis-inducing factor. Nature, **397,** 441-446 (1999).
Cai, J., et al., Mitochondrial control of apoptosis: the role of cytochrome c. Biochim. Biophys. Acta, **1366,** 139-149 (1998).
Lee, H., and Wei, Y., Mitochondrial role in life and death of the cell. J. Biomed. Sci., **7,** 2-15 (2000).

Mitochondrial Disease

- Mitochondrial disease is a difficult disorder to identify because it can take many forms, and range from mild to severe. The problems it causes may begin at birth or not occur until later in adult life. It is estimated that mitochondrial disease affects between 40,000 and 70,000 Americans, occurring in one in 2,500 to 4,000 births.
- **What is it?**
  Mitochondria (as many as 1,000 per cell). The mitochondria make the energy the cells need to grow and do their work in the body. If the mitochondria are damaged or malfunctioning, the cells cannot carry out their functions.
  - Mitochondria may not function correctly due to a genetic defect, damage caused by drugs, or damage caused by free radicals (destructive molecules).
- **Many effects, many symptoms**
  Because mitochondria are in cells all over the body, many different organs may be affected, including the brain and muscles. Some of the problems associated with mitochondrial disease are:
  - Brain: developmental delays, mental retardation, seizures, dementia
  - Nerves: weakness, pain
  - Muscles: weakness, low tone, cramping, pain
  - Heart disease
  - Eyes: twitching, vision loss
  - Kidney disease
  - Respiratory problems

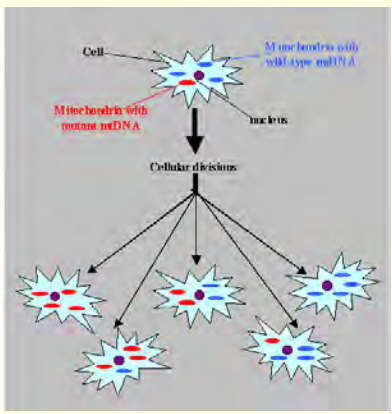
Mitochondrial Genetics

- ***Maternal inheritance***: Mitochondrial genome is inherited through the mother only (ovum). It allows to trace female lineage back in time.
- A few sperm mitochondria enter the egg, but they are degraded and lost.
- ***Mutation rate*** in mtDNA is very high: 10 times the nuclear rate. mtDNA is associated with the inner membrane, the site of oxidative phosphorylation. Large amounts of "reactive oxygen species" (peroxide and superoxide) are present, and they are quite mutagenic. Part of the effects of aging have been attributed to the gradual loss of mitochondria due to accumulated mutations in individual cells.



Heteroplasmy

- Sometimes an individual has more than one kind of mitochondria. This is called heteroplasmy. Since mitochondria are divided randomly during cell division, different cells get different proportions of the two types.
- If one mitochondrial type is mutant and the other is normal, severity of symptoms will vary in different tissues depending on the proportions of the two types.
- During oogenesis (egg formation), random segregation of the two types can lead to some offspring inheriting a mitochondrial disease while other offspring are normal.

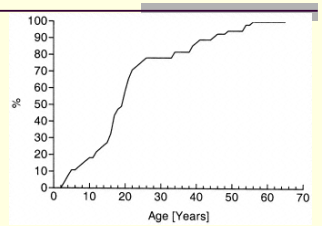


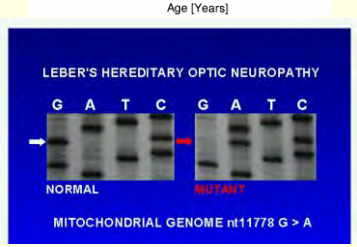
A three-parent baby

- http://nyti.ms/2dipoQm
- Mother with mutated mitochondria - *Leigh syndrome*
- The technique that led to the healthy birth was to move the DNA from an egg of the mother, who had mutated mitochondria, and place it in the egg of a healthy egg donor — after first removing the healthy donor's nuclear DNA from her egg

Genetic Diseases

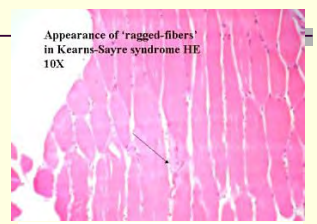
- In general: malfunctions of respiratory chain, so affects high metabolism tissues the most: nervous system, muscles, kidney, liver.
- https://www.ncbi.nlm.nih.gov/books/NBK1224/
- Leber's hereditary optic neuropathy (LHON).
  - Progressive loss of vision, from central to peripheral, usually beginning in 20's.
  - Eyes can be affected several months apart, or simultaneously.
  - About 85% are male (no good reason why).
  - Recurrance risk for siblings around 20% (heteroplasmy); many spontaneous cases.
  - Due to death of optic nerve fibers.
  - Most due to change in conserved Arg to His in NADH dehydrogenase, but 18 total mutations known, all missense in respiratory chain.



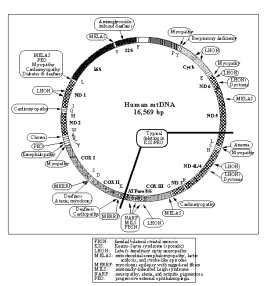


More Diseases

- Myoclonic epilepsy and ragged red fiber disease (MERRF). CNS symptoms: epilepsy, deafness, dementia. Skeletal and heart muscles abnormal, mitochondria appear abnormal. Multiple enzyme defects in respiratory chain. Lots of variation in inheritance of disease. Most due to A --> G in lysine tRNA (mutation A8344G). Easy to assay for; CviJI restriction site is altered. Good correlation between % mutant mitochondria and disease severity.
- Kearns-Sayre syndrome. paralysis of the muscles, retinal degeneration, cardiac muscle problems, seizures, many other symptoms irregularly. Due to large deletions of mtDNA. Heteroplasmy necessary for survival. Mostly spontaneous--rarely passed to offspring. Many variants.



Morbidity map of the human mitochondrial genome 1997



Genetic manipulation of mitochondrial fusion and fission proteins

Genetic manipulation of mitochondrial fusion and fission proteins in beta-cells and the resulting effect on mitochondrial morphology, mitochondrial function, and beta-cell function. Abbreviations: $\Delta\psi_{mt}$ – mitochondrial membrane potential; APs – autophagosomes; DN – dominant negative; GSIS – glucose-stimulated insulin secretion; KO – knockout; OEx – over-expression; ROS – reactive oxygen species.

	Manipulation	Model	Effect on morphology	Effect on mitochondrial function	Effect on beta-cell function	References
Pro-fusion	OPA1 OEx (low)	– INS-1 cells – Mouse islets	– Elongated (INS-1 cells) – Fragmentation (islets)	Decreased APs containing mitochondria (mitophagy) in INS-1 cells	N/A	Molina et al. (2009)[21] and Twig et al. (2008)[23]
	OPA1 OEx (high)	INS-1 cells	Fragmented	N/A	N/A	Molina et al. (2009)[21]
	Mfn1 OEx	INS-1 cells	Perinuclear aggregation of super-fused mitochondria	Decreased basal and glucose-stimulated ATP	Impaired GSIS	Park et al. (2008)[20]
	Fis1 RNAi	INS-1 cells	No effect	Decreased: – Maximal respiratory capacity – APs containing mitochondria (mitophagy)	Decreased GSIS	Molina et al. (2009)[21] and Twig et al. (2008)[23]
	Drp1-DN	– INS-1 cells – Mouse islets	– Super-fusion (INS-1 cells) – Swelling (islets)	Decreased: – Maximal respiratory capacity – APs containing mitochondria (mitophagy)	No statistically significant change in GSIS	Molina et al. (2009)[21]; Twig et al. (2008)[23]; Men et al. (2009)[25]
Pro-fission	OPA1 KO	Beta-cell specific mouse model	– Fragmented – Abnormal cristae structure	Decreased: – Glucose-stimulated ATP production – Glucose-stimulated O_2 consumption – ETC activity	Decreased GSIS	Zhang et al. (2011)[26]
	Mfn1-DN	INS-1 cells	Fragmented	No statistically significant changes	No effect on GSIS	Park et al. (2008)[20]
	hFis1 OEx	– INS-1 cells – Human beta-cells	Fragmented	Decreased: – Basal and glucose-stimulated ATP – Glucose-induced $\Delta\psi_{mt}$ hyperpolarization – Glucose-stimulated mitochondrial Ca^{2+}	Decreased: – Cytosolic Ca^{2+} release – GSIS	Park et al. (2008)[20]
	Drp1 OEx	INS-1 cells	Fragmented	– Increased cytochrome c expression and release – Increased ROS production	– No statistically significant change in GSIS; trend towards decreased GSIS – Increased apoptosis	Peng et al. (2011)[24] and Men et al. (2009)[25]

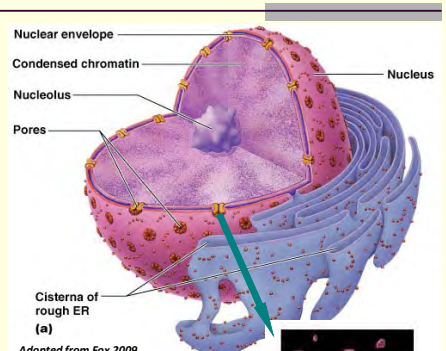
L. Sbiles, G.S. Shirihai / Best Practice & Research Clinical Endocrinology & Metabolism 26 (2012) 725–738

725

Cell Cycle
Apoptosis

Cell nucleus and genetic control

- Nucleus
  - most cells have single nucleus
  - contains genetic material
  - **controls all cell processes:**
    - Gene expression
    - Protein synthesis
    - Cell division
    - Cell death

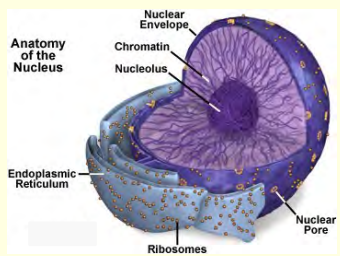


Adopted from Fox 2009


The Nuclear Pore Complex

Cell Nucleus Content

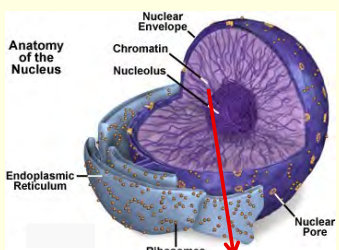
- The spherical nucleus typically occupies about 10% of a eukaryotic cell's volume. A double-layered membrane, the nuclear envelope, separates the contents of the nucleus from the cellular cytoplasm.
- The semi-fluid matrix found inside the nucleus is called *nucleoplasm*. Within the nucleoplasm, most of the nuclear material consists of chromatin, the less condensed form of the cell's DNA that organizes to form chromosomes during mitosis or cell division. The nucleus also contains one or more ***nucleoli***, organelles that synthesize protein-producing macromolecular assemblies called ribosomes, and a variety of other smaller components, such as *Cajal bodies*, **GEMS** (*Gemini of coiled bodies*), and ***interchromatin*** granule clusters.



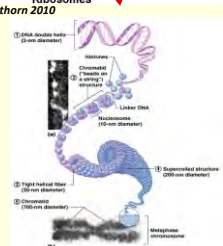
Adopted from Silverthorn 2010c

Cell Nucleus Content

- **Chromatin and Chromosomes** - Packed inside the nucleus of every human cell is nearly 1.83 m of DNA, which is divided into 46 individual molecules, one for each chromosome and each about 3.8 cm long. For DNA to function, it can't be crammed into the nucleus like a ball of string. Instead, it is combined with proteins and organized into a precise, compact structure, a dense string-like fiber called ***chromatin***.
- **The Nucleolus** - The nucleolus is a *membrane-less organelle* within the nucleus that manufactures ribosomes, the cell's protein-producing structures. Through the microscope, the nucleolus looks like a large dark spot within the nucleus. A nucleus may contain up to four nucleoli, but within each species the number of nucleoli is fixed. After a cell divides, a nucleolus is formed when chromosomes are brought together into nucleolar organizing regions. During cell division, the nucleolus disappears. Some studies suggest that the nucleolus may be involved with cellular aging and, therefore, may affect the senescence of an organism.
- **The Nuclear Envelope** - The nuclear envelope is a *double-layered* membrane that encloses the contents of the nucleus during most of the cell's lifecycle. The space between the layers is called the *perinuclear space* and appears to connect with the rough endoplasmic reticulum. The envelope is perforated with tiny holes called nuclear pores. The inner surface has a protein lining called the *nuclear lamina*, which binds to chromatin and other nuclear components. During mitosis, or cell division, the nuclear envelope disintegrates, but reforms as the two cells complete their formation and the chromatin begins to unravel and disperse.
- **Nuclear Pores** - The nuclear envelope is perforated with holes called nuclear pores that allow specific types and sizes of molecules to pass back and forth between the nucleus and the cytoplasm (gated transport see lecture *week 10*). Building blocks for building DNA and RNA are allowed into the nucleus as well as molecules that provide the energy for constructing genetic material.



Adopted from Silverthorn 2010



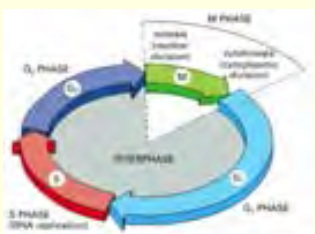
The Phases of the Cell Cycle

- Cell-cycle organization and control have been highly conserved during evolution, and studies in a wide range of systems—including yeasts, frog embryos, and mammalian cells in culture—have led to a unified view of eukaryotic cell-cycle control.
- Cell reproduction begins with duplication of the cell's contents, followed by distribution of those contents into two daughter cells.
- The most basic function of the cell cycle is to duplicate accurately the vast amount of DNA in the chromosomes and then segregate the copies precisely into two genetically identical daughter cells. These processes define the two major *phases* of the cell cycle.
  - Chromosome duplication occurs during ***S phase*** of the cell cycle, whereas most other cell components are duplicated continuously throughout the cycle.
  - During ***M phase***, the replicated chromosomes are segregated into individual nuclei (***mitosis***), and the cell then splits in two (***cytokinesis***).
  - S phase and M phase are usually separated by gap phases called G_1 and G_2, when cell-cycle progression can be regulated by various intracellular and extracellular signals.

Adopted from Alberts 2008

The Phases of the Cell Cycle

- Most cells require much more time to grow and double their mass of proteins and organelles than they require to replicate their DNA and divide. Partly to allow more time for growth, ***extra gap phases*** are inserted in most cell cycles—a ***G_1 phase*** between M phase and S phase and a ***G_2 phase*** between S phase and mitosis.
- Thus, the eukaryotic cell cycle is traditionally divided into four sequential phases: **G_1**, **S**, **G_2**, and **M**. G_1, S, and G_2 together are called ***interphase***.
- In a typical human cell proliferating in culture, ***interphase*** might occupy 23 hours of a 24 hour cycle, with 1 hour for M phase. The ***two gap phases*** serve as more than simple time delays to allow cell growth. They also ***provide time for the cell to monitor the internal and external environment***.
  - If extracellular conditions are unfavorable, for example, cells delay progress through G_1 and may even enter a specialized resting state known as ***G_0*** (**G zero**), in which they can remain for days, weeks, or even years before resuming proliferation. Indeed, many cells remain permanently in G_0 until they or the organism dies.
  - If extracellular conditions are favorable and signals to grow and divide are present, cells in early G_1 or G_0 progress through a commitment point near the end of G_1 known as **Start** (in yeasts) or the ***restriction point*** (in mammalian cells). After passing this point, cells are committed to DNA replication, even if the extracellular signals that stimulate cell growth and division are removed.

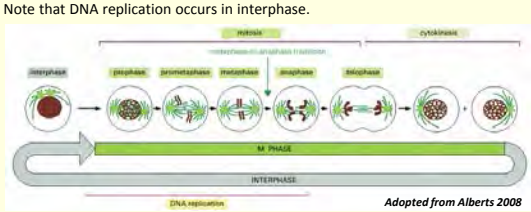


Adopted from Alberts 2008

An Overview of the Cell Cycle

- DNA duplication occurs during ***S phase*** (S for synthesis), which requires 10–12 hours and occupies about half of the cell-cycle time in a typical mammalian cell. After S phase, chromosome segregation and cell division occur in ***M phase*** (M for *m*itosis), which requires much less time (less than an hour in a mammalian cell).
- The easily visible processes of nuclear division (***mitosis***) and cell division (***cytokinesis***), collectively called ***M phase***, typically occupy only a small fraction of the cell cycle. The other, much longer, part of the cycle is known as ***interphase***. The five stages of mitosis are shown: an abrupt change in the biochemical state of the cell occurs at the transition from metaphase to anaphase. A cell can pause in metaphase before this transition point, but once the point has been passed, the cell carries on to the end of mitosis and through cytokinesis into interphase. Note that DNA replication occurs in interphase.

Adopted from Alberts 2008

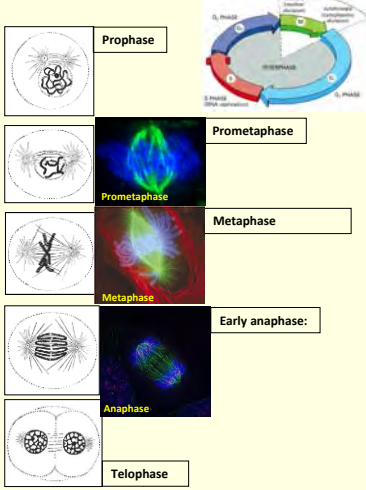


Adopted from Alberts 2008

An Overview of the Cell Cycle – M Phase

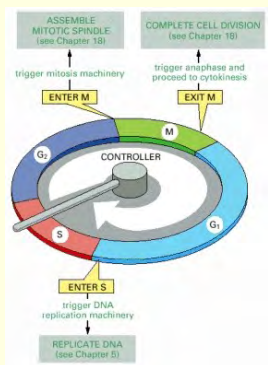
M phase - ***Mitosis***: prophase, prometaphase, metaphase, anaphase and telophase

- **Prophase** - Chromosome condensation: the duplicated DNA strands, packaged into elongated chromosomes, condense into the much more compact chromosomes required for their segregation. The two round objects above the nucleus are the centrosomes.
- **Prometaphase** - The nuclear envelope has degraded, and microtubules have invaded the nuclear space. These microtubules can attach to kinetochores or they can interact with opposing microtubules. The replicated chromosomes, each consisting of a pair of ***sister chromatids***, become attached to the microtubules of ***the mitotic spindle***.
- **Metaphase** - As mitosis proceeds, the cell pauses briefly in a state called *metaphase*, when the chromosomes are aligned at the equator of the mitotic spindle, poised for segregation.
- **Anaphase** - The sudden separation of sister chromatids marks the beginning of *anaphase*, during which the chromosomes move to opposite poles of the spindle, where they decondense and reform intact nuclei. Kinetochore microtubules shorten.
- **Telophase** - The decondensing chromosomes are surrounded by nuclear membranes. Note that cytokinesis has already begun, the pinching is known as the *cleavage furrow*. The cell is then pinched in two by cytoplasmic division, or ***cytokinesis***, and cell division is complete.



The Cell-Cycle Control System

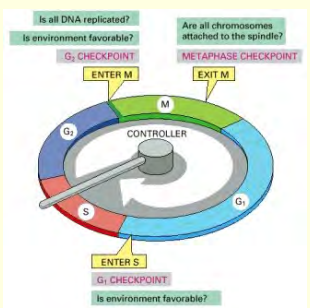
- *A clock*, or timer, that turns on each event at a specific time, thus providing a fixed amount of time for the completion of each event.
- *A mechanism for initiating events* in the correct order; entry into mitosis, for example, must always come after DNA replication.
- A mechanism to ensure that *each event is triggered only once per cycle*.
- *Binary (on/off) switches* that trigger events in a complete, irreversible fashion. It would clearly be disastrous, for example, if events like chromosome condensation or nuclear envelope breakdown were initiated but not completed.
- *Robustness:* backup mechanisms to ensure that the cycle can work properly even when parts of the system malfunction.
- *Adaptability*, so that the system's behavior can be modified to suit specific cell types or environmental conditions.



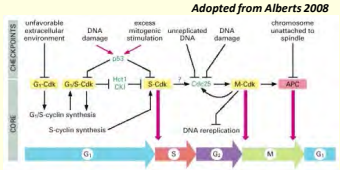
Adopted from Alberts 2008

The Control System Can Arrest the Cell Cycle at Specific Checkpoints

- In most cells there are several points in the cell cycle, called ***checkpoints***, at which the cycle can be arrested if previous events have not been completed.
  - Entry into mitosis is prevented, for example, when DNA replication is not complete, and chromosome separation in mitosis is delayed if some chromosomes are not properly attached to the mitotic spindle.
  - Progression through G_1 and G_2 is delayed by braking mechanisms if the DNA in the chromosomes is damaged by radiation or chemicals. Delays at these ***DNA damage checkpoints*** provide time for the damaged DNA to be repaired, after which the cell-cycle brakes are released and progress resumes.
- Checkpoints are important in another way as well. They are points in the cell cycle at which the control system can be regulated by extracellular signals from other cells. These signals—which can either promote or inhibit cell proliferation—tend to act by regulating progression through a G_1 checkpoint.



Adopted from Alberts 2008

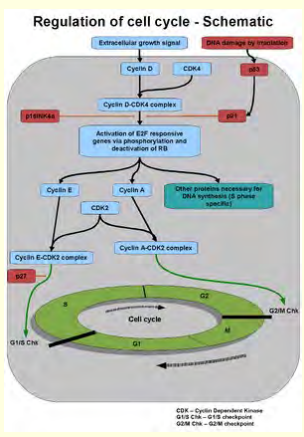


Regulation of eukaryotic cell cycle

- Regulation of the cell cycle involves processes crucial to the survival of a cell, including the detection and repair of genetic damage as well as the prevention of uncontrolled cell division. *The molecular events that control the cell cycle are ordered and unidirectional*; that is, each process occurs in a sequential fashion and it is impossible to "reverse" the cycle.

Role of cyclins and CDKs

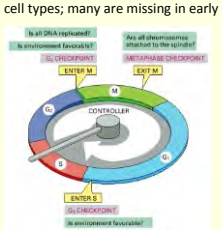
- Two key classes of regulatory molecules, ***cyclins*** and ***cyclin-dependent kinases*** (***CDKs***), determine a cell's progress through the cell cycle. ***Nobel Prize in Physiology in 2001*** was awarded for discovery of these central molecules.
- Many of the genes encoding cyclins and CDKs are conserved among all eukaryotes, but in general more complex organisms have more elaborate cell cycle control systems that incorporate more individual components. Many of the relevant genes were first identified by studying yeast.
- ***Cyclins*** form the ***regulatory subunits*** and ***CDKs*** ***the catalytic subunits*** of an activated heterodimer;
  - ***cyclins have no catalytic activity and CDKs are inactive in the absence of a partner cyclin***. When activated by a bound cyclin, CDKs perform a common biochemical reaction called phosphorylation that activates or inactivates target proteins to orchestrate coordinated entry into the next phase of the cell cycle.
- Different cyclin-CDK combinations determine the downstream proteins targeted. CDKs are constitutively expressed in cells whereas cyclins are synthesised at specific stages of the cell cycle, in response to various molecular signals.



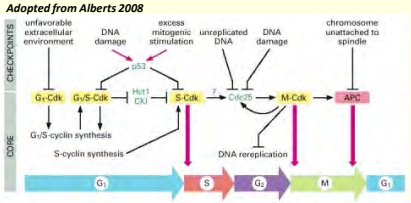
Adopted from Alberts 2008

Regulation of eukaryotic cell cycle

- An ordered sequence of cyclin-Cdk activities triggers most of the events of the cell cycle. During G_1 phase, Cdk activity is reduced to a minimum by Cdk inhibitors (CKIs), cyclin proteolysis, and decreased cyclin gene transcription. When environmental conditions are favorable, G_1- and G_1/S-Cdks increase in concentration, overcoming these inhibitory barriers in late G_1 and triggering the activation of S-Cdk. The S-Cdk phosphorylates proteins at DNA replication origins, initiating DNA synthesis through a mechanism that ensures that the DNA is duplicated only once per cell cycle.
- Once S phase is completed, the activation of M-Cdk leads to the events of early mitosis, whereby the cell assembles a mitotic spindle and prepares for segregation of the duplicated chromosomes—which consist of sister chromatids glued together. Anaphase is triggered by the destruction of the proteins that hold the sisters together. The M-Cdk is then inactivated by cyclin proteolysis, which leads to cytokinesis and the end of M phase. Progression through the cell cycle is regulated precisely by various inhibitory mechanisms that arrest the cell cycle at specific checkpoints when events are not completed successfully, when DNA damage occurs, or when extracellular conditions are unfavorable.
- The *core of the cell-cycle control system* consists of a *series of cyclin-Cdk complexes* ***(yellow boxes)***. The activity of each complex is also influenced by various inhibitory checkpoint mechanisms, which provide information about the extracellular environment, cell damage, and incomplete cell-cycle events *(top)*. These mechanisms are not present in all cell types; many are missing in early embryonic cell cycles, for example.

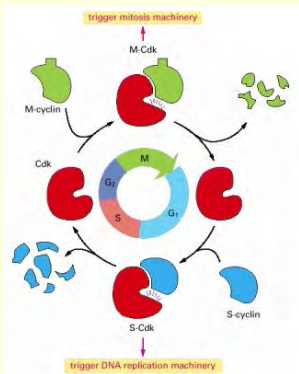


Adopted from Alberts 2008



The Cell-Cycle Control System Is Based on Cyclically Activated Protein Kinases

- At the heart of the cell-cycle control system is a family of protein kinases known as ***cyclin-dependent kinases (Cdks)***. The activity of these kinases rises and falls as the cell progresses through the cycle. ***The oscillations lead directly to cyclical changes in the phosphorylation of intracellular proteins*** that initiate or regulate the major events of the cell cycle—DNA replication, mitosis, and cytokinesis. An increase in Cdk activity at the beginning of mitosis, for example, leads to increased phosphorylation of proteins that control chromosome condensation, nuclear envelope breakdown, and spindle assembly.
- Cyclical changes in Cdk activity are controlled by a complex array of enzymes and other proteins. The most important of these Cdk regulators are proteins known as ***cyclins***. Cdks, as their name implies, are dependent on cyclins for their activity: unless they are tightly bound to a cyclin, they have no protein kinase activity
- ***Cyclins*** were originally named as such because they undergo a cycle of synthesis and degradation in each cell cycle. ***Cdk levels***, by contrast, are constant, at least in the simplest cell cycles. Cyclical changes in cyclin levels result in the cyclic assembly and activation of ***the cyclin-Cdk complexes***; this activation in turn triggers cell-cycle events



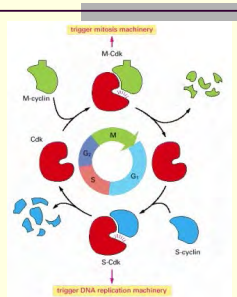
Adopted from Alberts 2008

The Cell-Cycle Control System Is Based on Cyclically Activated Protein Kinases

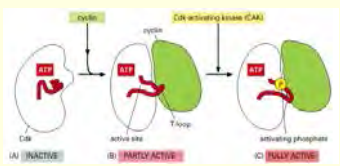
Four classes of cyclins:

1) **G_1/S-cyclins** bind Cdks at the end of G_1 and commit the cell to DNA replication.
2) **S-cyclins** bind Cdks during S phase and are required for the initiation of DNA replication.
3) **M-cyclins** promote the events of mitosis.
4) **G_1-cyclins** help promote passage through Start or the restriction point in late G_1.

- ***Three-dimensional structure of Cdk:***
  - In the absence of cyclin, the active site in the Cdk protein is partly obscured by a slab of protein, like a stone blocking the entrance to a cave
  - Cyclin binding causes the slab to move away from the active site, resulting in partial activation of the Cdk enzyme.
  - Full activation of the cyclin-Cdk complex then occurs when a separate kinase, the **Cdk-activating kinase (CAK)**, phosphorylates an amino acid near the entrance of the Cdk active site. This causes a small conformational change that further increases the activity of the Cdk, allowing the kinase to phosphorylate its target proteins effectively and thereby induce specific cell-cycle events.

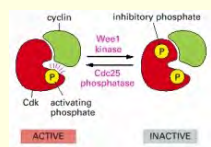


Adopted from Alberts 2008

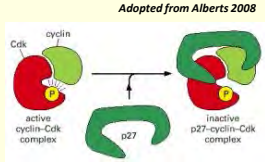


Cdk Activity Can Be Suppressed Both by Inhibitory Phosphorylation and by Inhibitory Proteins

- The rise and fall of cyclin levels is the primary determinant of Cdk activity during the cell cycle. Several additional mechanisms, however, are important for fine-tuning Cdk activity at specific stages in the cell cycle.
- The activity of a cyclin-Cdk complex can be inhibited by phosphorylation at a pair of amino acids in the roof of the active site. Phosphorylation of these sites by a protein kinase known as ***Wee1*** **inhibits Cdk activity**, while dephosphorylation of these sites by a phosphatase known as **Cdc25 increases Cdk activity**
  - The active cyclin-Cdk complex is turned off when the kinase Wee1 phosphorylates two closely spaced sites above the active site. Removal of these phosphates by the phosphatase Cdc25 results in activation of the cyclin-Cdk complex. For simplicity, only one inhibitory phosphate is shown. The activating phosphate is added by CAK
- Cyclin-Cdk complexes can also be regulated by the binding of ***Cdk inhibitor proteins (CKIs)***. There are a variety of CKI proteins, and they are primarily employed in the control of G_1 and S phase. The three-dimensional structure of a cyclin-Cdk-CKI complex reveals that CKI binding dramatically rearranges the structure of the Cdk active site, rendering it inactive

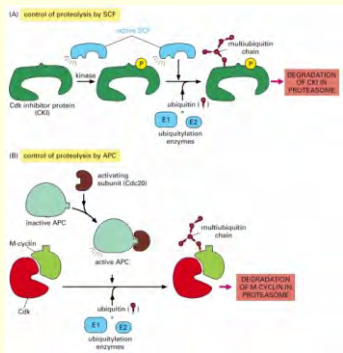


Adopted from Alberts 2008



The Cell-Cycle Control System Depends on Cyclical Proteolysis

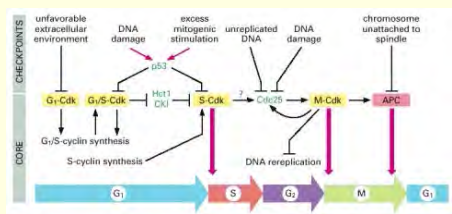
- Cell-cycle control depends crucially on at least **two distinct enzyme complexes** that act at different times in the cycle to cause the proteolysis of key proteins of the cell-cycle control system, thereby inactivating them. The cyclin destruction occurs by a ***ubiquitin-dependent mechanism***, like that involved in the proteolysis of many other intracellular proteins. An activated enzyme complex recognizes specific amino-acid sequences on the cyclin and attaches multiple copies of ubiquitin to it, marking the protein for complete destruction in proteasomes.
- The **rate-limiting step** in cyclin destruction is the final ubiquitin-transfer reaction catalyzed by enzymes known as ***ubiquitin ligases.*** **Two** ubiquitin ligases are important in the destruction of cyclins and other cell-cycle regulators.
  - In G_1 and S phase, an enzyme complex called ***SCF*** (after its three main protein subunits) is responsible for the ubiquitylation and destruction of G_1/S-cyclins and certain CKI proteins that control S-phase initiation.
  - In M phase, the *anaphase-promoting complex* (***APC***) is responsible for the ubiquitylation and proteolysis of M-cyclins and other regulators of mitosis.



Adopted from Alberts 2008

Intracellular Control of Cell-Cycle Events

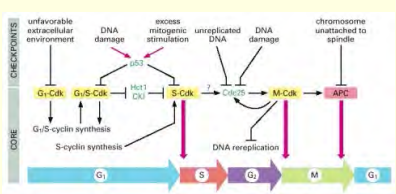
- Each of the different cyclin-Cdk complexes serves as a molecular switch that triggers a specific cell-cycle event.
- The two central events of the cell cycle:
  - the replication of DNA during S phase and the chromosome segregation and cell division of M phase.
  - how crucial regulatory mechanisms in G_1 phase control whether or not a cell proliferates.

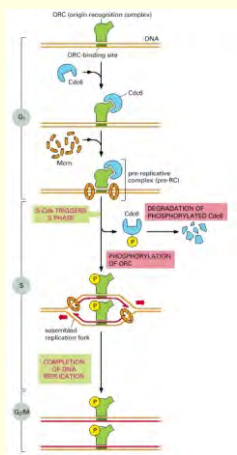


Adopted from Alberts 2008

S-Phase Cyclin-Cdk Complexes (S-Cdks) Initiate DNA Replication Once Per Cycle

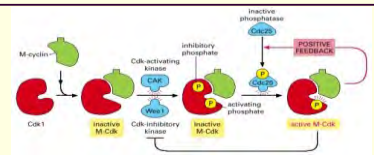
- DNA replication begins at ***origins of replication***, which are scattered at various locations in the chromosome. Replication origins are simple and well defined in the budding yeast *S. cerevisiae*, and most of our understanding of the initiation machinery comes from studies of this organism. Analyses of proteins that bind to the yeast replication origin have identified a large, multiprotein complex known as the **origin recognition complex (ORC)**. These complexes bind to replication origins throughout the cell cycle and serve as landing pads for several additional regulatory proteins.
- One of these regulatory proteins is **Cdc6**. It is present at low levels during most of the cell cycle but increases transiently in early G_1. It binds to ORC at replication origins in early G_1, where it is required for the binding of a complex composed of a group of closely related proteins, the *Mcm proteins*. The resulting large protein complex formed at an origin is known as the **pre-replicative complex**, or **pre-RC** . Once the pre-RC has been assembled in G_1, the replication origin is ready to fire. The activation of S-Cdk in late G_1 pulls the trigger and initiates DNA replication. The initiation of replication also requires the activity of a second protein kinase, which collaborates with S-Cdk to cause the phosphorylation of ORC.
- The S-Cdk not only initiates origin firing, but also helps to prevent re-replication.





Adopted from Alberts 2008

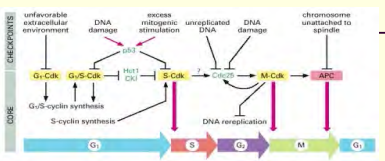
The Activation of M-Phase Cyclin-Cdk Complexes (M-Cdks) Triggers Entry into Mitosis



Adopted from Alberts 2008

- The completion of DNA replication leaves the G_2 cell with two accurate copies of the entire genome, with each replicated chromosome consisting of two identical ***sister chromatids*** glued together along their length. The cell then undergoes the dramatic upheaval of M phase, in which the duplicated chromosomes and other cell contents are distributed equally to the two daughter cells. The events of mitosis are triggered by M-Cdk, which is activated after S phase is complete.
- The ***activation of M-Cdk begins with the accumulation of M-cyclin*** (cyclin B in vertebrate cells). In embryonic cell cycles, the synthesis of M-cyclin is constant throughout the cell cycle, and M-cyclin accumulation results from a decrease in its degradation. In most cell types, however, M-cyclin synthesis increases during G_2 and M, owing primarily to an increase in *M-cyclin* gene transcription. This increase in M-cyclin protein leads to a gradual accumulation of M-Cdk (the complex of Cdk1 and M-cyclin) as the cell approaches mitosis. Although the Cdk in these complexes is phosphorylated at an activating site by the enzyme CAK discussed earlier, it is held in an inactive state by inhibitory phosphorylation at two neighboring sites by the protein kinase Wee1. Thus, by the time the cell reaches the end of G_2, it contains an abundant stockpile of M-Cdk that is primed and ready to act, but the M-Cdk activity is repressed by the presence of two phosphate groups that block the active site of the kinase.
- The ability of M-Cdk to activate its own activator (Cdc25) and inhibit its own inhibitor (Wee1) suggests that **M-Cdk activation in mitosis involves a positive feedback loop**. According to this attractive model, the partial activation of Cdc25, leads to the partial activation of a subpopulation of M-Cdk complexes, which then phosphorylate more Cdc25 and Wee1 molecules. This leads to more M-Cdk dephosphorylation and activation, and so on. Such a mechanism would quickly promote the complete activation of all the M-Cdk complexes in the cell, converting a gradual increase in M-cyclin levels into a switchlike, abrupt rise in M-Cdk activity. As mentioned earlier, similar molecular switches operate at various points in the cell cycle to ensure that events such as entry into mitosis occur in an all-or-none fashion.

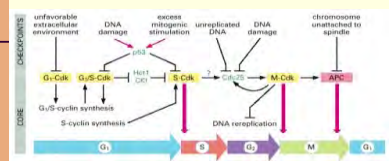
Entry into Mitosis Is Blocked by Incomplete DNA Replication: The DNA Replication Checkpoint



Adopted from Alberts 2008

- If a cell is driven into mitosis before it has finished replicating its DNA, it will pass on broken or incomplete sets of chromosomes to its daughter cells. This disaster is avoided in most cells by a ***DNA replication checkpoint*** mechanism, which ensures that ***the initiation of mitosis cannot occur until the last nucleotide in the genome has been copied***. Sensor mechanisms, of unknown molecular nature, detect either the unreplicated DNA or the corresponding unfinished replication forks and send a negative signal to the cell-cycle control system, blocking the activation of M-Cdk. Thus, normal cells treated with chemical inhibitors of DNA synthesis, such as hydroxyurea, do not progress into mitosis.
  - The final targets of the negative checkpoint signal are the enzymes that control M-Cdk activation. The negative signal activates a protein kinase that inhibits the Cdc25 protein phosphatase
  - As a result, M-Cdk remains phosphorylated and inactive until DNA replication is complete.
- One of the most remarkable features of cell-cycle control is that a single protein kinase, M-Cdk, is able to bring about all of the diverse and complex rearrangements that occur in the early stages of mitosis.
  - At a minimum, M-Cdk must induce the ***assembly of the mitotic spindle*** and ensure that replicated chromosomes attach to the spindle. In many organisms, M-Cdk also ***triggers chromosome condensation***, ***nuclear envelope breakdown***, ***actin cytoskeleton rearrangement***, and the ***reorganization of the Golgi apparatus and endoplasmic reticulum***. Each of these events is thought to be triggered when M-Cdk phosphorylates specific structural or regulatory proteins involved in the event, although most of these proteins have not yet been identified.
- Phosphorylation by M-Cdk also triggers the complex microtubule rearrangements and other events that lead to the assembly of the mitotic spindle. M-Cdk is known to phosphorylate a number of proteins that regulate microtubule behavior, causing the increase in microtubule instability that is required for spindle assembly.

Spindle-attachment checkpoint

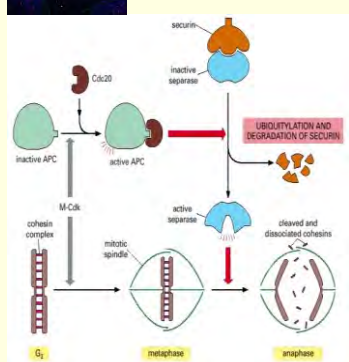


Adopted from Alberts 2008

- **Unattached Chromosomes Block Sister-Chromatid Separation**: **The Spindle-Attachment Checkpoint** - The cell does not commit itself to the momentous events of anaphase before it is fully prepared. In most cell types, a spindle-attachment checkpoint mechanism operates to ensure that all chromosomes are properly attached to the spindle before sister-chromatid separation occurs. The checkpoint depends on a sensor mechanism that monitors the state of the *kinetochore,* the specialized region of the chromosome that attaches to microtubules of the spindle. Any kinetochore that is not properly attached to the spindle sends out a negative signal to the cell-cycle control system, blocking Cdc20-APC activation and sister-chromatid separation.
- **Exit from Mitosis Requires the Inactivation of M-Cdk**
  - After the chromosomes have been segregated to the poles of the spindle, the cell must reverse the complex changes of early mitosis. The spindle must be disassembled, the chromosomes decondensed, and the nuclear envelope reformed. Because the phosphorylation of various proteins is responsible for getting cells into mitosis in the first place, it is not surprising that the dephosphorylation of these same proteins is required to get them out. In principle, these dephosphorylations and the exit from mitosis could be triggered by the inactivation of M-Cdk, the activation of phosphatases, or both. Evidence suggests that M-Cdk inactivation is primarily responsible.
  - M-Cdk inactivation occurs mainly by ubiquitin-dependent proteolysis of M-cyclins. Ubiquitylation of the cyclin is usually triggered by the same Cdc20-APC complex that promotes the destruction of Securin at the metaphase-to-anaphase transition

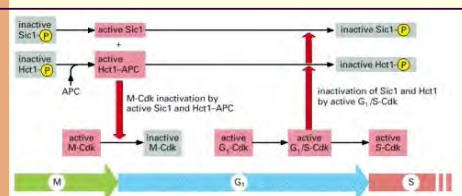
Sister Chromatid Separation Is Triggered by Proteolysis

- After M-Cdk has triggered the complex rearrangements that occur in early mitosis, the cell cycle reaches its culmination with the separation of the **sister chromatids** at the *metaphase-to-anaphase transition.* Although M-Cdk activity sets the stage for this event, an entirely different enzyme complex—the ***anaphase-promoting complex (APC)*** introduced earlier—throws the switch that initiates sister-chromatid separation.
- The attachment of the two sister chromatids to opposite poles of the mitotic spindle early in mitosis results in forces tending to pull the two chromatids apart. These pulling forces are initially resisted because the sister chromatids are bound tightly together, both at their centromeres and all along their arms. This sister-chromatid cohesion depends on a complex of proteins, the ***cohesin*** **complex**, that is deposited along the chromosomes as they are duplicated in S phase.
- Anaphase begins with a sudden disruption of the cohesion between sister chromatids, which allows them to separate and move to opposite poles of the spindle. This process is initiated by a remarkable cascade of signaling events. The sister-chromatid separation requires the **activation of the APC enzyme complex**, suggesting that proteolysis is central to the process. The relevant target of the APC is the protein ***securin***. Before anaphase, securin binds to and inhibits the activity of a protease called ***separase***
- If the APC triggers anaphase, what triggers the APC? The answer is only partly known. APC activation requires the protein **Cdc20**, which binds to and activates the APC at mitosis.

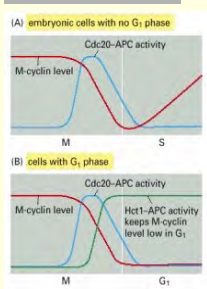


Adopted from Alberts 2008

The G_1 Phase Is a State of Stable Cdk Inactivity

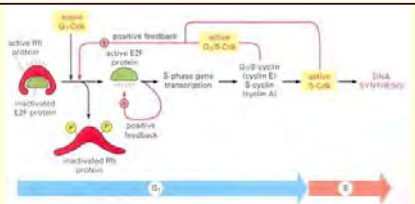


Adopted from Alberts 2008



- In early animal embryos, ***the inactivation of M-Cdk in late mitosis is due*** almost entirely ***to the action of Cdc20-APC***. Thus, the destruction of M-cyclin in late mitosis soon leads to the inactivation of all APC activity in an embryonic cell. This is a useful arrangement in rapid embryonic cell cycles, as APC inactivation immediately after mitosis allows the cell to quickly begin accumulating new M-cyclin for the next cycle.
- Rapid cyclin accumulation immediately after mitosis is not useful, however, in cell cycles containing a G_1 phase. In these cycles, progression into the next S phase is delayed in G_1 to allow for cell growth and for the cycle to be regulated by extracellular signals. Thus, most cells employ several mechanisms to ensure that Cdk reactivation is prevented after mitosis. One mechanism makes use of another APC-activating protein called *Hct1,* a close relative of Cdc20.
- How does the cell escape from this stable G_1 state to initiate S phase? escape usually occurs through the accumulation of G_1-cyclins.
  - In animal cells, the accumulation of G_1-cyclins is stimulated by the extracellular signals that promote cell proliferation

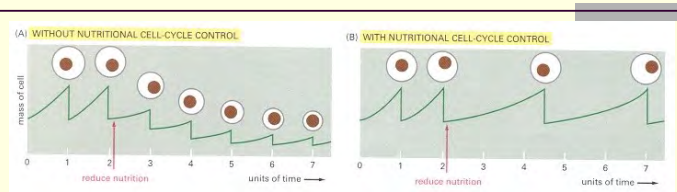
The Rb Protein Acts as a Brake in Mammalian G_1 Cells



Adopted from Alberts 2008

- ***The control of G_1 progression and S-phase initiation is often disrupted in cancer cells***, leading to unrestrained cell-cycle entry and cell proliferation . To develop improved methods for controlling cancer growth, we need a better understanding of the proteins that control G_1 progression in mammalian cells.
- Animal cells suppress Cdk activity in G_1 by the same three mechanisms as budding yeast: **Hct1 activation**, **the accumulation of a CKI protein** (p27 in mammalian cells), and **the inhibition of cyclin gene transcription**. As in yeasts, the activation of G_1-Cdk complexes reverses all three inhibitory mechanisms in late G_1.
- The best understood effects of G_1-Cdk activity in animal cells are mediated by a **gene regulatory protein called E2F**. It binds to specific DNA sequences in the promoters of many genes that encode proteins required for S-phase entry, including G_1/S-cyclins and S-cyclins. E2F function is controlled primarily by an interaction with the ***retinoblastoma protein (Rb)***, ***an inhibitor of cell-cycle progression***. During G_1, Rb binds to E2F and blocks the transcription of S-phase genes. When cells are stimulated to divide by extracellular signals, active G_1-Cdk accumulates and phosphorylates Rb, reducing its affinity for E2F. The Rb then dissociates, allowing E2F to activate S-phase gene expression.
- This transcriptional control system, like so many other control systems that regulate the cell cycle, includes **positive feedback loops** that sharpen the G_1/S transition:
  - The liberated E2F increases the transcription of its own gene.
  - E2F-dependent transcription of G_1/S-cyclin and S-cyclin genes leads to increased G_1/S-Cdk and S-Cdk activities, which in turn increase Rb phosphorylation and promote further E2F release.
  - The increase in G_1/S-Cdk and S-Cdk activities enhances the phosphorylation of Hct1 and p27, leading to their inactivation or destruction.

Cell-Cycle Progression Is Somehow Coordinated With Cell Growth

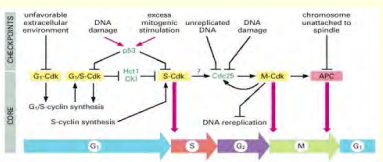


Adopted from Alberts 2008

- For proliferating cells to maintain a relatively constant size, the length of the cell cycle must match the time it takes the cell to double in size. If the cycle time is shorter than this, the cells will get smaller with each division; if it is longer, the cells will get bigger with each division. Because cell growth depends on nutrients and growth signals in the environment, the length of the cell cycle has to be able to adjust to varying environmental conditions . It is not clear how proliferating cells coordinate their growth with the rate of cell-cycle progression to maintain their size.
- The size at which an animal cell divides depends, at least in part, on these extracellular signals, which can regulate cell growth and proliferation independently. Animal cells can also completely uncouple cell growth and division so as to grow without dividing or to divide without growing. The eggs of many animals, for example, grow to an extremely large size without dividing. After fertilization, this relationship is reversed, and many rounds of division occur without growth. Thus, although cell growth and cell division are usually coordinated, they can be regulated independently.

Cell-Cycle Progression is Blocked by DNA Damage and p53: DNA Damage Checkpoints

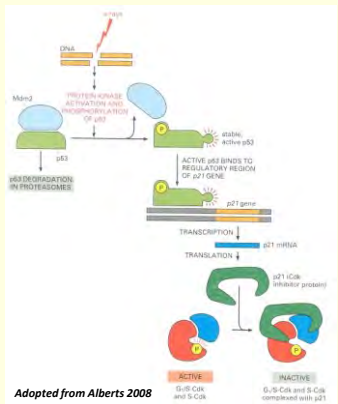
- When chromosomes are damaged, as can occur after exposure to radiation or certain chemicals, it is essential that they be repaired before the cell attempts to duplicate or segregate them.
- The cell-cycle control system can readily detect DNA damage and arrest the cycle at ***DNA damage checkpoints***. Most cells have at least **two such checkpoints -** one in **late G_1 (p53)**, which prevents entry into S phase, and one in **late G_2(Cdc25)**, which prevents entry into mitosis.
- The G_2 checkpoint depends on a mechanism similar to the one discussed earlier that delays entry into mitosis in response to incomplete DNA replication. When cells in G_2 are exposed to damaging radiation, for example, the damaged DNA sends a signal to a series of protein kinases that phosphorylate and inactivate the phosphatase Cdc25. This blocks the dephosphorylation and activation of M-Cdk, thereby blocking entry into mitosis. When the DNA damage is repaired, the inhibitory signal is turned off, and cell-cycle progression resumes.



Adopted from Alberts 2008

Cell-Cycle Progression is Blocked by DNA Damage and p53: DNA Damage Checkpoints

- The **G_1 checkpoint** blocks progression into S phase by inhibiting the activation of G_1/S-Cdk and S-Cdk complexes. In mammalian cells, for example, DNA damage leads to the activation of the gene regulatory protein **p53**, which stimulates the transcription of several genes. One of these genes encodes a CKI protein called **p21**, which binds to G_1/S-Cdk and S-Cdk and inhibits their activities, thereby helping to block entry into S phase.
- DNA damage activates p53 by an indirect mechanism. In undamaged cells, p53 is highly unstable and is present at very low concentrations. This is because it interacts with another protein, **Mdm2**, that acts as a ubiquitin ligase that targets p53 for destruction by proteasomes. DNA damage activates protein kinases that phosphorylate p53 and thereby reduce its binding to Mdm2. This decreases p53 degradation, which results in a marked increase in p53 concentration in the cell. In addition, the decreased binding to Mdm2 enhances the ability of p53 to stimulate gene transcription
- This loss of p53 function allows the cancer cell to accumulate mutations more readily. Similarly, a rare genetic disease known as ***ataxia telangiectasia*** is caused by a defect in one of the protein kinases that phosphorylates and activates p53 in response to x-ray-induced DNA damage; patients with this disease are very sensitive to x-rays due to the loss of the DNA damage checkpoints, and they consequently suffer from increased rates of cancer.



Adopted from Alberts 2008

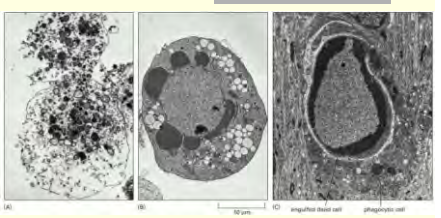
Cell Death

- What if DNA damage is so severe that repair is not possible?
  - In this case, the response is different in different organisms.
- Unicellular organisms such as budding yeast transiently arrest their cell cycle to repair the damage. If repair cannot be completed, the cycle resumes despite any damage. For a single-celled organism, life with mutations is apparently better than no life at all.
- In multicellular organisms, however, the health of the organism takes precedence over the life of an individual cell. Cells that divide with severe DNA damage threaten the life of the organism, since genetic damage can often lead to cancer and other lethal defects.
  - Thus, animal cells with severe DNA damage do not attempt to continue division, but instead commit suicide by undergoing programmed cell death, or apoptosis. *The decision to die in this way also depends on the activation of p53, and it is this function of p53 that is apparently most important in protecting us against cancer.*

Cell Death

Type of Cell Death:

- Cells that die as a result of acute injury typically swell and burst.
- ***Necrosis*** - cells spill their contents all over their neighborscausing a potentially damaging inflammatory response.
- ***Apoptosis*** - By contrast, a cell that undergoes apoptosis dies neatly, without damaging its neighbors. The cell shrinks and condenses. The cytoskeleton collapses, the nuclear envelope disassembles, and the nuclear DNA breaks up into fragments. Most importantly, the cell surface is altered, displaying properties that cause the dying cell to be rapidly phagocytosed, either by a neighboring cell or by a macrophage, before any leakage of its contents occurs. This not only avoids the damaging consequences of cell necrosis but also allows the organic components of the dead cell to be recycled by the cell that ingests it.

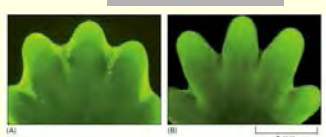


Adopted from Alberts 2008

- These electron micrographs show cells that have died by (**A**) necrosis or (**B** and **C**) apoptosis. The cells in (A) and (B) died in a culture dish, whereas the cell in (C) died in a developing tissue and has been engulfed by a neighboring cell. Note that the cell in (A) seems to have exploded, whereas those in (B) and (C) have condensed but seem relatively intact. The large vacuoles visible in the cytoplasm of the cell in (B) are a variable feature of apoptosis.

Programmed Cell Death (Apoptosis)

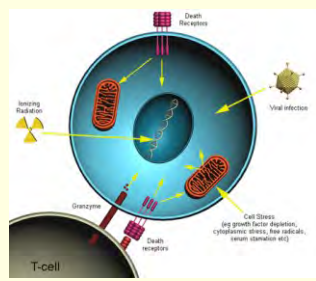
- The cells of a multicellular organism are members of a highly organized community. The number of cells in this community is tightly regulated—not simply by controlling the rate of cell division, but also by controlling the rate of cell death. If cells are no longer needed, they commit suicide by activating an intracellular death program. This process is therefore called ***programmed cell death***, although it is more commonly called ***apoptosis*** (from a Greek word meaning "falling off," as leaves from a tree).
- The amount of apoptosis that occurs in developing and adult animal tissues can be astonishing.
  - In the developing vertebrate nervous system, for example, up to half or more of the nerve cells normally die soon after they are formed.
  - In a healthy adult human, billions of cells die in the bone marrow and intestine every hour.
- It seems remarkably wasteful for so many cells to die, especially as the vast majority are perfectly healthy at the time they kill themselves. ***What purposes does this massive cell death serve?***
- In adult tissues, ***cell death exactly balances cell division***. If this were not so, the tissue would grow or shrink.
  - If part of the liver is removed in an adult rat, for example, liver cell proliferation increases to make up the loss. Conversely, if a rat is treated with the drug phenobarbital—which stimulates liver cell division (and thereby liver enlargement)—and then the phenobarbital treatment is stopped, apoptosis in the liver greatly increases until the liver has returned to its original size, usually within a week or so. Thus, the liver is kept at a constant size through the regulation of both the cell death rate and the cell birth rate.



(A) The paw in this mouse embryo has been stained with a dye that specifically labels cells that have undergone apoptosis. The apoptotic cells appear as *bright green* dots between the developing digits. (B) This interdigital cell death eliminates the tissue between the developing digits, as seen one day later, when few, if any, apoptotic cells can be seen. (From W. Wood et al., *Development* 127:5245–5252, 2000)

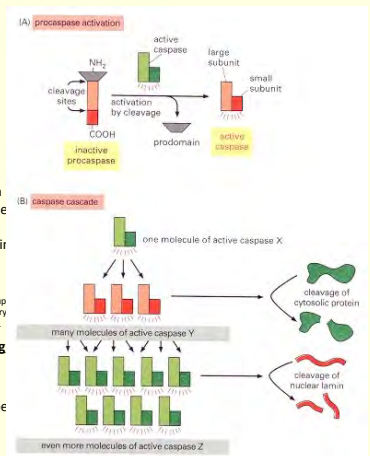
Triggers Responsible for Apoptosis

- There are a number of mechanisms through which apoptosis can be induced in cells. The sensitivity of cells to any of these stimuli can vary depending on a number of factors such as:
  - The expression of pro- and anti-apoptotic proteins (eg. the Bcl-2 proteins or the Inhibitor of Apoptosis Proteins),
  - The severity of the stimulus and the stage of the cell cycle
- In some cases the apoptotic stimuli comprise ***extrinsic signals*** such as the binding of death inducing ligands to cell surface receptors called ***death receptors***.
  - These ligands can either be soluble factors or can be expressed on the surface of cells such as cytotoxic T lymphocytes. The latter occurs when T-cells recognise damaged or virus infected cells and initiate apoptosis in order to prevent damaged cells from becoming neoplastic (cancerous) or virus-infected cells from spreading the infection.
- In other cases apoptosis can be initiated following ***intrinsic signals*** that are produced following cellular stress.
  - Cellular stress may occur from ***exposure to radiation or chemicals*** or to ***viral infection***. It might also be a consequence of ***growth factor deprivation*** or ***oxidative stress caused by free radicals***.
- In general intrinsic signals initiate apoptosis via the involvement of the mitochondria. The relative ratios of the various Bcl-2 proteins can often determine how much cellular stress is necessary to induce apoptosis.



Apoptosis Is Mediated by an Intracellular Proteolytic Cascade

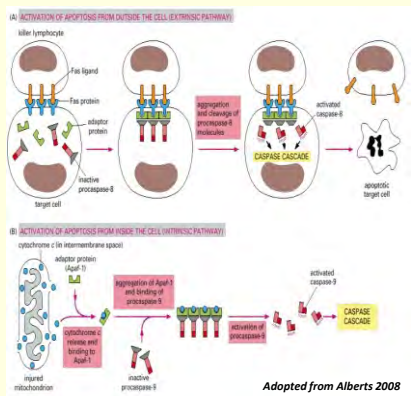
- The caspase cascade involved in apoptosis
- This machinery depends on a family of proteases that have a cysteine at their active site and cleave their target proteins at specific aspartic acids. They are therefore called ***caspases***.
- Caspases are synthesized in the cell as inactive precursors, or ***procaspases***, which are usually activated by cleavage at aspartic acids by other caspases.
- Once activated, caspases cleave, and thereby activate, other procaspases, resulting in an amplifying proteolytic cascade.
- Some of the activated caspases then cleave other key proteins in the cell. Some cleave the nuclear lamins, for example, causing the irreversible breakdown of the nuclear lamina; another cleaves a protein that normally holds a DNA-degrading enzyme (a DNAse) in an inactive form, freeing the DNAse to cut up the DNA in the cell nucleus.
  - In this way, the cell dismantles itself quickly and neatly, and its corpse is rapidly taken up and digested by another cell. Activation of the intracellular cell death pathway, like entry into a new stage of the cell cycle, is usually triggered in a complete, all-or-none fashion.
- **The protease cascade is not only destructive and self-amplifying but also irreversible**, so that once a cell reaches a critical point along the path to destruction, it cannot turn back.
- The intracellular machinery responsible for apoptosis seems to be similar in all animal cells



Adopted from Alberts 2008

Procaspases Are Activated by Binding to Adaptor Proteins

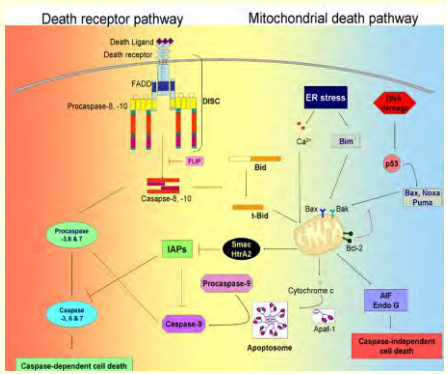
- All nucleated animal cells contain the seeds of their own destruction, in the form of various inactive procaspases that lie waiting for a signal to destroy the cell. It is therefore not surprising that caspase activity is tightly regulated inside the cell to ensure that the death program is held in check until needed.
- How are procaspases activated to initiate the caspase cascade? A general principle is that the activation is triggered by *adaptor proteins* that bring multiple copies of specific procaspases, known as ***initiator procaspases***, close together in a complex or aggregate. In some cases, the initiator procaspases have a small amount of protease activity, and forcing them together into a complex causes them to cleave each other, triggering their mutual activation. In other cases, the aggregation is thought to cause a conformational change that activates the procaspase. Within moments, the activated caspase at the top of the cascade cleaves downstream procaspases to amplify the death signal and spread it throughout the cell.
- Procaspase activation can be triggered from outside the cell by the activation of ***death receptors*** on the cell surface.
- When cells are damaged or stressed, they can also kill themselves by triggering procaspase aggregation and activation from within the cell.



Adopted from Alberts 2008

Bcl-2 Family Proteins and IAP Proteins Are the Main Intracellular Regulators of the Cell Death Program

- The ***Bcl-2 family*** of intracellular proteins helps regulate the activation of procaspases. Some members of this family, like *Bcl-2* itself or *Bcl-X_L*, inhibit apoptosis, at least partly by blocking the release of cytochrome *c* from mitochondria. Other members of the Bcl-2 family are not death inhibitors, but instead promote procaspase activation and cell death. Some of these apoptosis promoters, such as *Bad*, function by binding to and inactivating the death-inhibiting members of the family, whereas others, like *Bax* and *Bak,* stimulate the release of cytochrome *c* from mitochondria. If the genes encoding Bax and Bak are both inactivated, cells are remarkably resistant to most apoptosis-inducing stimuli, indicating the crucial importance of these proteins in apoptosis induction. Bax and Bak are themselves activated by other apoptosis-promoting members of the Bcl-2 family such as Bid.
- Another important family of intracellular apoptosis regulators is the ***IAP (inhibitor of apoptosis) family***. These proteins are thought to inhibit apoptosis in two ways: they bind to some procaspases to prevent their activation, and they bind to caspases to inhibit their activity. IAP proteins were originally discovered as proteins produced by certain insect viruses, which use them to prevent the infected cell from killing itself before the virus has had time to replicate. When mitochondria release cytochrome *c* to activate Apaf-1, they also release a protein that blocks IAPs, thereby greatly increasing the efficiency of the death activation process.
- The intracellular cell death program is also regulated by extracellular signals, which can either activate apoptosis or inhibit it. These signal molecules mainly act by regulating the levels or activity of members of the Bcl-2 and IAP families. We see in the next section how these signal molecules help multicellular organisms regulate their cell numbers.



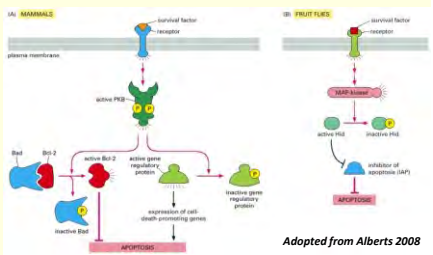
Adopted from Gupta et al. (2009)

Extracellular Control of Cell Division, Cell Growth, and Apoptosis

- The size of an organ or organism depends mainly on its total cell mass, which depends on both the total number of cells and the size of the cells. Cell number, in turn, depends on the amounts of cell division and cell death. Organ and body size are therefore determined by three fundamental processes: cell growth, cell division, and cell death. Each is independently regulated—both by intracellular programs and by extracellular signal molecules that control these programs.
- The extracellular signal molecules that regulate cell size and cell number are generally either soluble secreted proteins, proteins bound to the surface of cells, or components of the extracellular matrix. The factors that promote organ or organism growth can be operationally divided into three major classes:
  1) ***Mitogens***, which stimulate cell division, primarily by relieving intracellular negative controls that otherwise block progress through the cell cycle.
  2) ***Growth factors***, which stimulate cell growth (an increase in cell mass) by promoting the synthesis of proteins and other macromolecules and by inhibiting their degradation.
  3) ***Survival factors***, which promote cell survival by suppressing apoptosis.
- Some extracellular signal molecules promote all of these processes, while others promote one or two of them. Indeed, the term *growth factor* is often used inappropriately to describe a factor that has any of these activities. Even worse, the term *cell growth* is often used to mean an increase in cell number, or *cell proliferation.*

Extracellular Survival Factors Suppress Apoptosis

- Animal cells need signals from other cells—not only to grow and proliferate, but also to survive. If deprived of such ***survival factors***, cells activate their intracellular death program and die by apoptosis. This arrangement ensures that cells survive only when and where they are needed.
  - Nerve cells, for example, are produced in excess in the developing nervous system and then compete for limited amounts of survival factors that are secreted by the target cells they contact.
  - Nerve cells that receive enough survival factor live, while the others die by apoptosis. A similar dependence on survival signals from neighboring cells is thought to control cell numbers in other tissues, both during development and in adulthood.
- Survival factors, just like ***mitogens*** and ***growth factors***, usually bind to cell-surface receptors. Binding activates signaling pathways that keep the death program suppressed, often by regulating members of the Bcl-2 family of proteins. Some factors, for example, stimulate the increased production of apoptosis-suppressing members of this family. Others act by inhibiting the function of apoptosis-promoting members of the family.



Adopted from Alberts 2008

Two ways in which survival factors suppress apoptosis

(A) In mammalian cells, the binding of some survival factors to cell-surface receptors leads to the activation of various protein kinases, including protein kinase B (PKB), that phosphorylate and inactivate the Bcl-2 family member Bad. When not phosphorylated, Bad promotes apoptosis by binding and inhibiting Bcl-2. Once phosphorylated, Bad dissociates, freeing Bcl-2 to suppress apoptosis. As indicated, PKB also suppresses death by phosphorylating and thereby inhibiting gene regulatory proteins of the Forkhead family that stimulate the transcription of genes that encode proteins that promote apoptosis.

(B) In *Drosophila*, some survival factors inhibit apoptosis by stimulating the phosphorylation of the Hid protein. When not phosphorylated, Hid promotes cell death by inhibiting IAPs. Once phosphorylated, Hid no longer inhibits IAPs, which become active and block cell death.

References

1. Adams, Francis (1891), *The Genuine Works of Hippocrates* New York: William Wood and Company.
2. Alberts B., A. Johnson, J. Lewis, M. Raff, K. Roberts, P. Walter (2008). Molecular Biology of the Cell, 5th Edition, Garland Science
3. Anderson & Greenberg (2001) Comp.Biochem.Physiol. Part B 129: 17-18
4. Avicenna (Persian philosopher and scientist) - Britannica Online Encyclopedia". Britannica.com.
5. Bereiter-Hahn J and Voth M. (1994) Microscopy Research and Techniques 27: 198–219
6. Butterfield, H.(1957). The Origins of Modern Science (revised ed.). New York: The Free Press.
7. Cleland et al. (2011) Bcl-2 family interaction with the mitochondrial morphogenesis machinery. Cell Death and Differentiation 18: 235–247
8. Cotterill R.M.J. (2002) Biophysics – An Introduction, J.Wiley & Sons,Ltd.
9. Dear, P. (2001) Revolutionizing the Sciences: European Knowledge and Its Ambitions, 1500-1700. Princeton: Princeton UP,
10. Faccenda et al. (2013) Mitochondrial IF_1 preserves cristae structure to limit apoptotic cell death signaling. Cell Cycle 12(16) 2530–2532
11. Fox, Stuart Ira (2009) Human Physiology, 11th Edition, McGraw Hill
12. Garrison, Fielding H. (1966) *History of Medicine*, Philadelphia: W.B. Saunders Company.
13. Gupta et al. (2009) The mitochondrial death pathway: a promising therapeutic target in diseases J. Cell. Mol. Med. Vol 13, No 6, 2009 pp. 1004-1033
14. Hille B. (2001) Ion channels of excitable membranes, 3rd Edition, Sinauer Associates, Inc.
15. Hirokawa, N. (1998) Kinesin and Dynein Superfamily Proteins and the Mechanism of Organelle Transport
16. *Science* 279:519-526
17. Iskandar, Albert (2006). "Al-Rāzī". *Encyclopedia of the history of science, technology, and medicine in non-western cultures* (2nd ed.). Springer. pp. 155–156.
18. Jackson M.B. (2006) Molecular and Cellular Biophysics, Cambridge Univ. Press
19. Karmažínová M. and Lacinová L. (2010) Measurement of Cellular Excitability by Whole Cell Patch Clamp
20. Technique *Physiol. Res. 59 (Suppl. 1): S1-S7*
21. Karnkowska A., et al. (2016) A Eukaryote without a Mitochondrial Organelle. Current Biology 26(10) p1274–1284, 23
22. Kearney, H. (1971). *Science and Change 1500 – 1700*. New York: McGraw-Hill.
23. Krauss G. (2003) Biochemistry of Signal Transduction and Regulation, Wiley/VCH
24. Lodish H, Berk A, Zipursky SL, et al. (2000) Molecular Cell Biology. 4th edition. New York: W. H. Freeman;
25. Mackinnon, Roderick (2004) Potassium channels and the atomic basis of selective ion conduction (Nobel lecture). Angew. Chem. Int. Ed., 43:4265–4277, 2004.
26. Marbán E., (2002) Cardiac channelopathies Nature 415, 213-218 doi:10.1038/415213a
27. Nicholls D.G. and S.J.Ferguson (2013) Bioenergetics, Academic Press, 4th Ed.
28. Nutton V. (1984) From Galen to Alexander, aspects of medicine and medical practice in late antiquity. Dunbarton Oaks Papers. 38,
29. Osellame L.D. et al. (2012) Best Practice & Research Clinical Endocrinology & Metabolism 26: 711–723
30. Otera H, Ishihara N., and Mihara K. (2013) New insights into the function and regulation of mitochondrial fission Biochimica et Biophysica Acta 1833: 1256–1268
31. Rambold & Lippincott-Swarz (2011) Mechanisms of mitochondria and autophagy crosstalk. Cell Cycle 10 (23): 4032-4038
32. Richards et al. (1998) Phylogeography of mitochondrial DNA in western Europe. *Ann. Hum. Genet.*) 62:241-260
33. Rizzuto R. *et al.* (1998) Close Contacts with the Endoplasmic Reticulum as Determinants of Mitochondrial Ca^{2+} Responses**.** *Science* 280: 1763 – 1766
34. Schmidt and Mangelsdorf (2008) Nuclear receptors of the enteric tract: guarding the frontier. Nutr Rev. 2008 October ; 66(10 Suppl 2): S88–S97
35. Shi Y. (2007) Orphan nuclear receptors in drug discovery. Drug Discov Today. 12(11-12): 440–445. doi:10.1016/j.drudis.2007.04.006.
36. Silverthorn D.U.(2010) Human Physiology – An Integrated Approach, Pearson-Benjamin Cummings
37. Sorrenson, Richard (1996). "Towards a History of the Royal Society in the Eighteenth Century". *Notes and Records of the Royal Society of London* (The Royal Society) **50** (1): 29. doi:10.1098/rsnr.1996.0003
38. Staden, Heinrich von (1989). *Herophilus: The Art of Medicine in Early Alexandria: Edition, Translation and Essays*. Cambridge University Press. p. 138. ISBN 9780521236461
39. Stiles L., and Shirihai O.S. (2012) Mitochondrial dynamics and morphology in beta-cells. Best Practice & Research Clinical Endocrinology & Metabolism 26:725–738
40. Wood et al. (2000) Mesenchymal cells engulf and clear apoptotic footplate cells in macrophageless PU.1 null mouse embryo. *Development* 127:5245–5252,

Cell Biophysics II

Vysokoškolský učebný text

Autorka:	RNDr. Katarína Stroffeková, Ph.D.
Vydavateľ:	Univerzita Pavla Jozefa Šafárika v Košiciach
Umiestnenie:	http://unibook.upjs.sk/
Rok vydania:	2017
Dostupné od:	23. 03. 2017
Rozsah strán:	257
Vydanie:	prvé

ISBN 978-80-8152-494-3