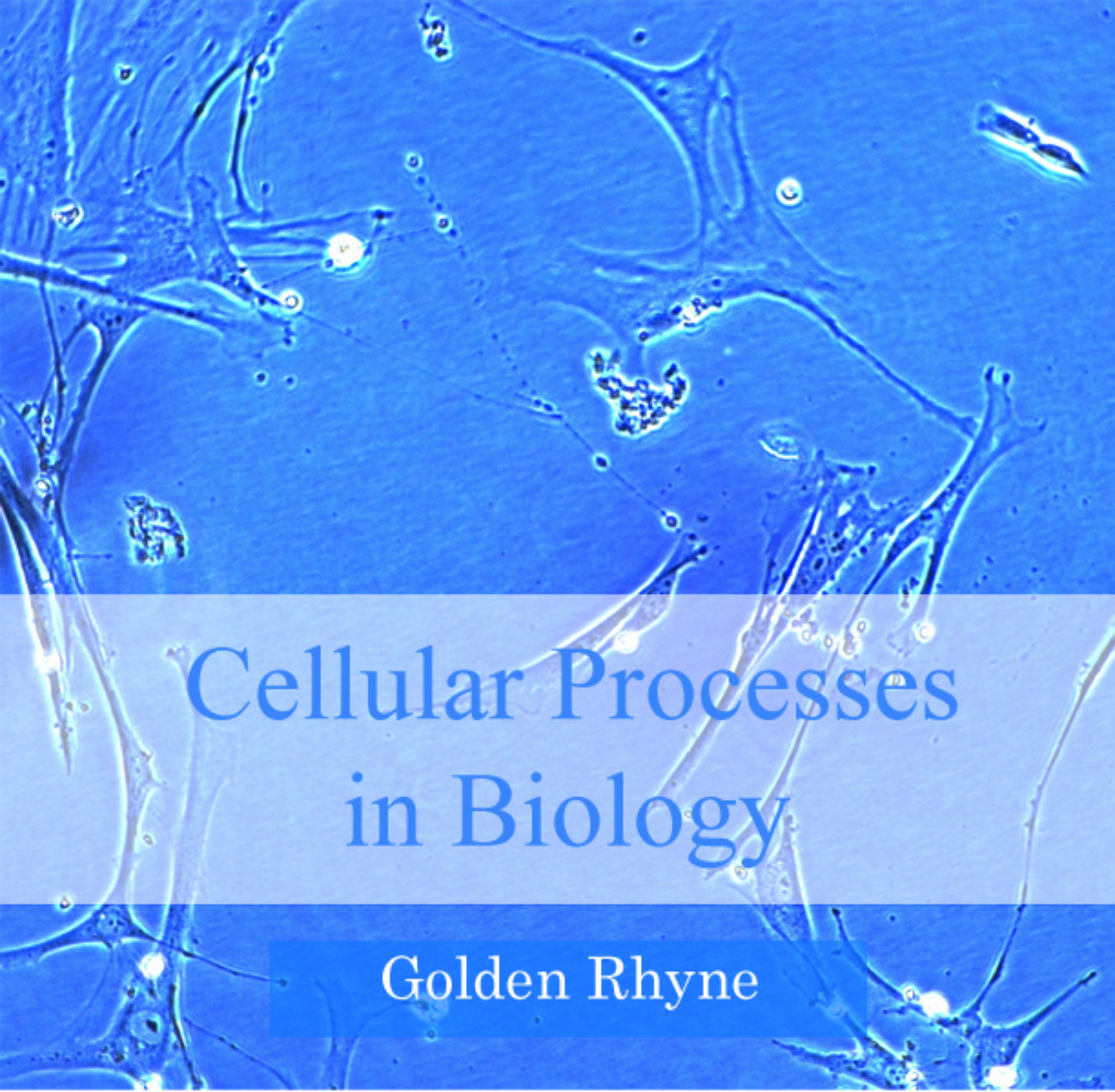

Cellular Processes in Biology

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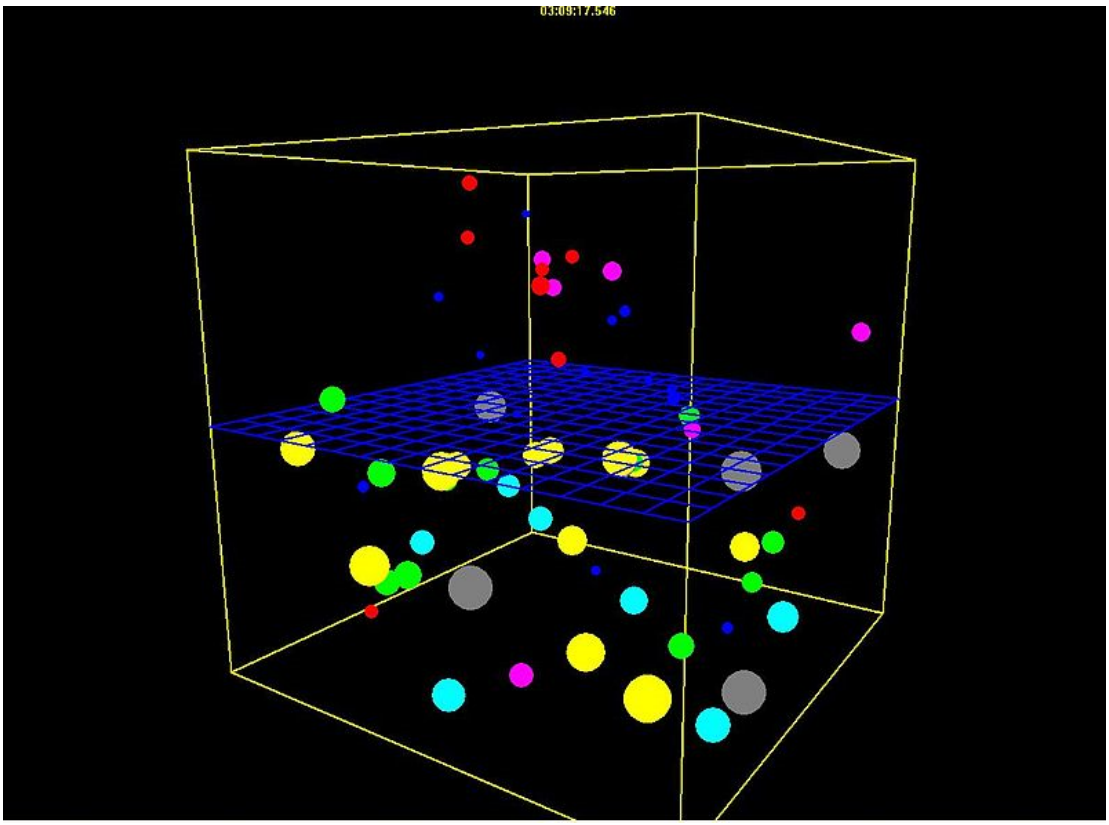
Chapter- 1

Osmosis

Osmosis is the movement of water molecules through a selectively-permeable membrane down a water potential gradient. More specifically, it is the movement of water across a selectively permeable membrane from an area of high water potential (low solute concentration) to an area of low water potential (high solute concentration). It may also be used to describe a physical process in which any solvent moves, without input of energy, across a semipermeable membrane (permeable to the solvent, but not the solute) separating two solutions of different concentrations. Osmosis releases energy, and can be made to do work, but is a passive process, like diffusion.



Shot of a computer simulation of the process of osmosis

Net movement of solvent is from the less-concentrated (*hypotonic*) to the more-concentrated (*hypertonic*) solution, which tends to reduce the difference in concentrations. This effect can be countered by increasing the pressure of the hypertonic solution, with respect to the hypotonic. The osmotic pressure is defined to be the pressure required to maintain an equilibrium, with no net movement of solvent. Osmotic pressure is a colligative property, meaning that the osmotic pressure depends on the molar concentration of the solute but not on its identity.

Osmosis is important in biological systems, as many biological membranes are semipermeable. In general, these membranes are impermeable to organic solutes with large molecules, such as polysaccharides, while permeable to water and small, uncharged solutes. Permeability may depend on solubility properties, charge, or chemistry, as well as solute size. Water molecules travel through the plasma cell wall, tonoplast (vacuole) or protoplast in two ways, either by diffusing across the phospholipid bilayer directly, or via aquaporins (small transmembrane proteins similar to those in facilitated diffusion and in creating ion channels). Osmosis provides the primary means by which water is transported into and out of cells. The turgor pressure of a cell is largely maintained by osmosis, across the cell membrane, between the cell interior and its relatively hypotonic environment.

The first recorded observation of osmosis was in 1748 by Jean-Antoine Nollet. The word "osmosis" descends from the words "endosmose" and "exosmose", which were coined by French physician René Joachim Henri Dutrochet (1776-1847) from the Greek words ένδον (*endon* : within), έξο (*exo* : outside), and ωσμος (*osmos* : push, impulsion).

Basic explanations

Osmosis may occur when there is a partially permeable membrane, such as a cell membrane. Impermeable means that the object doesn't allow things through it, so a membrane has to be permeable so food gets in and waste gets out. When a cell is submerged in water, the water molecules pass through the cell membrane from an area of low solute concentration (outside the cell) to one of high solute concentration (inside the cell); this is called osmosis. The cell membrane is selectively permeable, so only necessary materials are let into the cell and wastes are left out.

When the membrane has a volume of pure water on both sides, water molecules pass in and out in each direction at exactly the same rate; there is no net flow of water through the membrane.

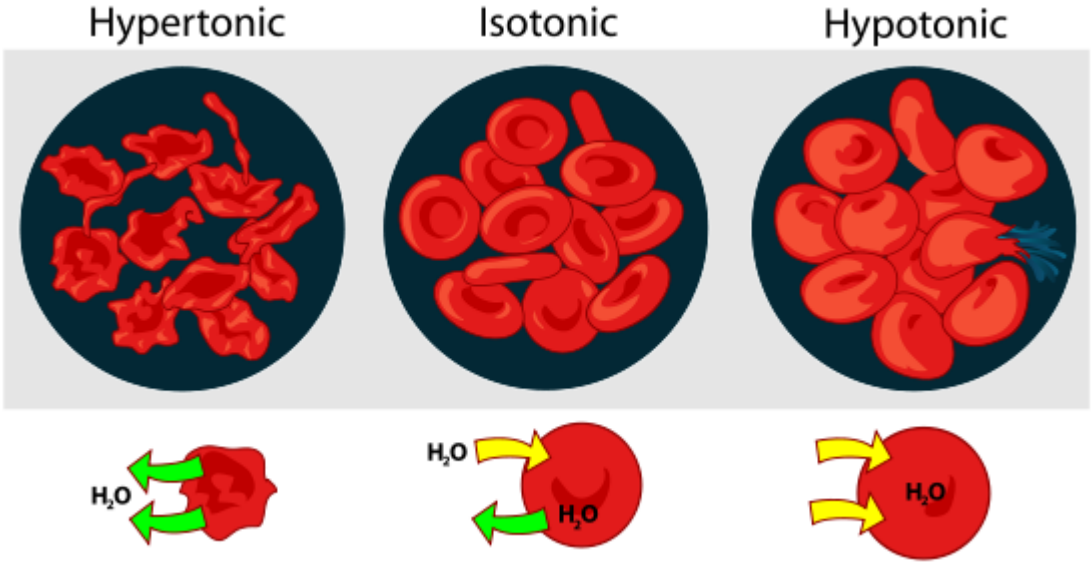
In a solution, the water is diluted (i.e., the concentration of water is lowered) by the presence of solute particles. If there is a solution on one side, and pure water on the other, there will be a higher concentration of water molecules on the pure water side of the membrane. Therefore, water molecules pass through the membrane from the pure water side toward the solution side more frequently than from the solution side going to the pure water side. This will result in a net flow of water to the side with the solution. Assuming the membrane does not break, this net flow will slow and finally stop as the

pressure on the solution side becomes such that the movement in each direction is equal: dynamic equilibrium. This could either be due to the water potential on both sides of the membrane being the same, or due to osmosis being inhibited by factors such as pressure potential or osmotic pressure.

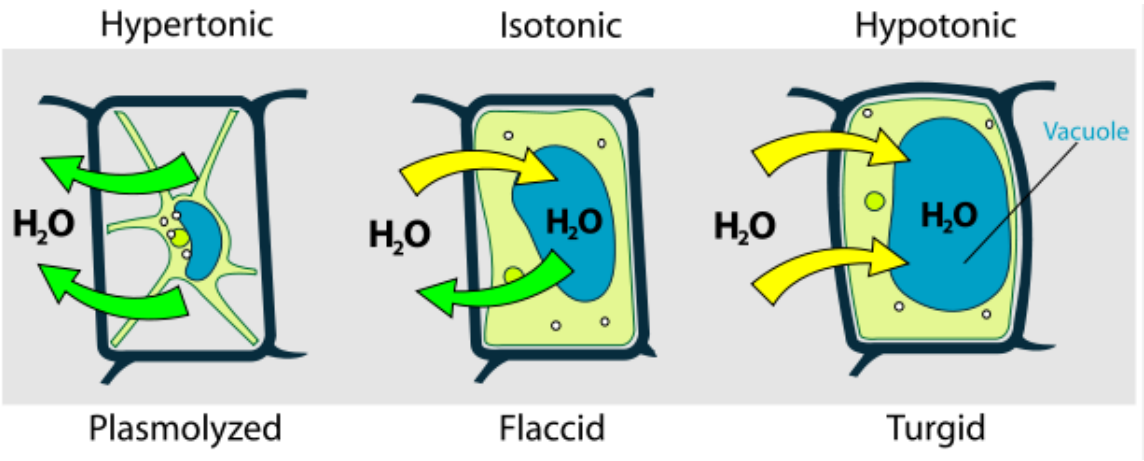
Osmosis can also be explained using the notion of entropy, from statistical mechanics. Suppose a permeable membrane separates equal amounts of pure solvent and a solution. Since a solution possesses more entropy than pure solvent, the second law of thermodynamics states that solvent molecules will flow into the solution until the entropy of the combined system is maximized. Notice that, as this happens, the solvent loses entropy while the solution gains entropy. Equilibrium, hence maximum entropy, is achieved when the entropy gradient becomes zero, and dissolution takes place.

Pure water is more ordered than water in a solution; thus, from an entropic standpoint it takes some net energy to move a water molecule from a disordered solution and "pack it in" with pure water. This is the same explanation as to why the disordered air does not spontaneously separate and order into oxygen and nitrogen, it would take energy for this to happen. Additionally, particle size has no bearing on osmotic pressure, as this is the fundamental postulate of colligative properties.

Examples of osmosis



Effect of different solutions on blood cells



Plant cell under different environments

Osmotic pressure is the main cause of support in many plants. The osmotic entry of water raises the turgor pressure exerted against the cell wall, until it equals the osmotic pressure, creating a steady state.

When a plant cell is placed in a hypertonic solution, the water in the cells moves to an area higher in solute concentration and the cell shrinks, and in doing so, becomes *flaccid*. This means the cell has become plasmolysed - the cell membrane has completely left the cell wall due to lack of water pressure on it; the opposite of *turgid*.

Also, osmosis is responsible for the ability of plant roots to draw water from the soil. Since there are many fine roots, they have a large surface area, and water enters the roots by osmosis.

Osmosis can also be seen when potato slices are added to a high concentration of salt solution. The water from inside the potato moves to the salt solution, causing the potato to shrink and to lose its 'turgor pressure'. The more concentrated the salt solution, the bigger the difference in size and weight of the potato slice.

In unusual environments, osmosis can be very harmful to organisms. For example, freshwater and saltwater aquarium fish placed in water of a different salinity than that to which they are adapted to will die quickly, and in the case of saltwater fish, dramatically. Another example of a harmful osmotic effect is the use of table salt to kill leeches and slugs.

Suppose an animal or a plant cell is placed in a solution of sugar or salt in water.

1. If the medium is *hypotonic* — a dilute solution, with a higher water concentration than the cell — the cell will gain water through osmosis.
2. If the medium is *isotonic* — a solution with exactly the same water concentration as the cell — there will be no net movement of water across the cell membrane.

3. If the medium is *hypertonic* — a concentrated solution, with a lower water concentration than the cell — the cell will lose water by osmosis.

Essentially, this means that if a cell is put in a solution which has a solute concentration higher than its own, then it will shrivel up, and if it is put in a solution with a lesser solute concentration than its own, the cell will expand and burst. *Electronucleal exchange* is the passive diffusion of cations and anions across a semi-permeable membrane according to electrical charge.

Chemical gardens demonstrate the effect of osmosis in inorganic chemistry.

Factors

Osmotic pressure

As mentioned before, osmosis may be opposed by increasing the pressure in the region of high solute concentration with respect to that in the low solute concentration region. The force per unit area, or pressure, required to prevent the passage of water through a selectively permeable membrane and into a solution of greater concentration is equivalent to the osmotic pressure of the solution, or turgor. Osmotic pressure is a colligative property, meaning that the property depends on the concentration of the solute, but not on its identity.

Osmotic gradient

The osmotic gradient is the difference in concentration between two solutions on either side of a semipermeable membrane, and is used to tell the difference in percentages of the concentration of a specific particle dissolved in a solution.

Usually the osmotic gradient is used while comparing solutions that have a semipermeable membrane between them allowing water to diffuse between the two solutions, toward the hypertonic solution (the solution with the higher concentration). Eventually, the force of the column of water on the hypertonic side of the semipermeable membrane will equal the force of diffusion on the hypotonic (the side with a lesser concentration) side, creating equilibrium. When equilibrium is reached, water continues to flow, but it flows both ways in equal amounts as well as force, therefore stabilizing the solution.

Variation

Reverse osmosis

Reverse osmosis is a separation process that uses pressure to force a solvent through a semipermeable membrane that retains the solute on one side and allows the pure solvent to pass to the other side. More formally, it is the process of forcing a solvent from a

region of high solute concentration through a membrane to a region of low solute concentration by applying a pressure in excess of the osmotic pressure.

Forward osmosis

Osmosis may be used directly to achieve separation of water from a "feed" solution containing unwanted solutes. A "draw" solution of higher osmotic pressure than the feed solution is used to induce a net flow of water through a semipermeable membrane, such that the feed solution becomes concentrated as the draw solution becomes dilute. The diluted draw solution may then be used directly (as with an ingestible solute like glucose), or sent to a secondary separation process for the removal of the draw solute. This secondary separation can be more efficient than a reverse osmosis process would be alone, depending on the draw solute used and the feedwater treated. Forward osmosis is an area of ongoing research, focusing on applications in desalination, water purification, water treatment, food processing, etc.

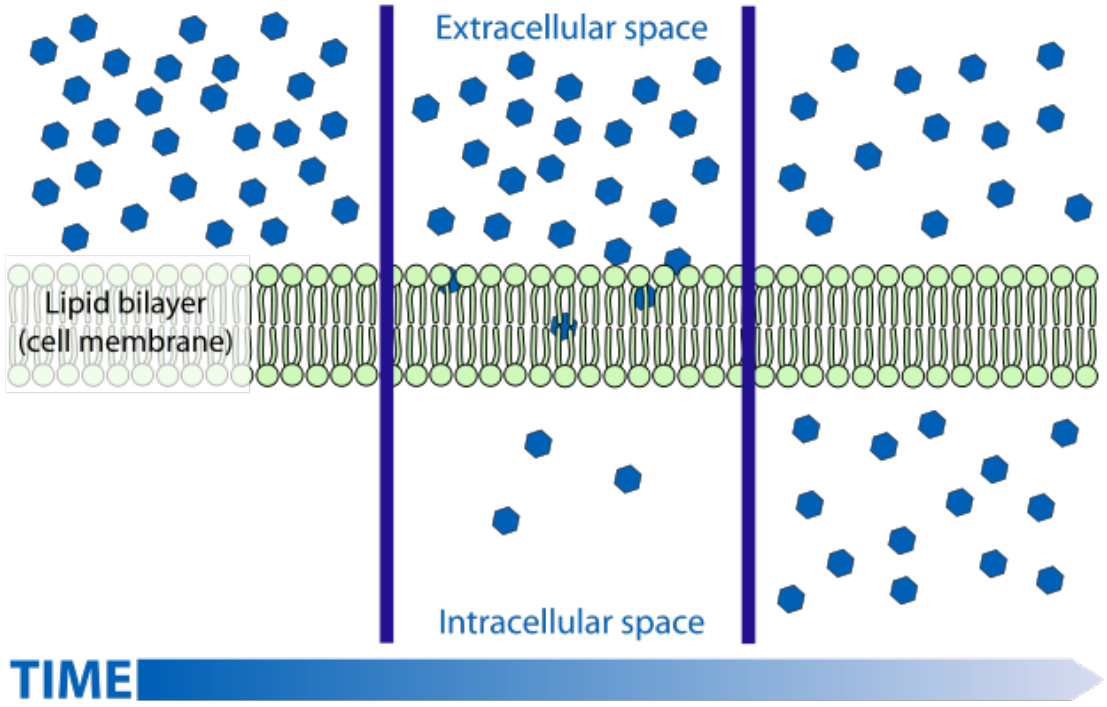
Chapter- 2

Passive Transport and Active Transport

Passive transport

Passive transport means moving biochemicals and other atomic or molecular substances across membranes. Unlike active transport, this process does not involve chemical energy, because, unlike in an active transport, the transport across membrane is always coupled with the growth of entropy of the system. So passive transport is dependent on the permeability of the cell membrane, which, in turn, is dependent on the organization and characteristics of the membrane lipids and proteins. The four main kinds of passive transport are diffusion, facilitated diffusion, filtration and osmosis.

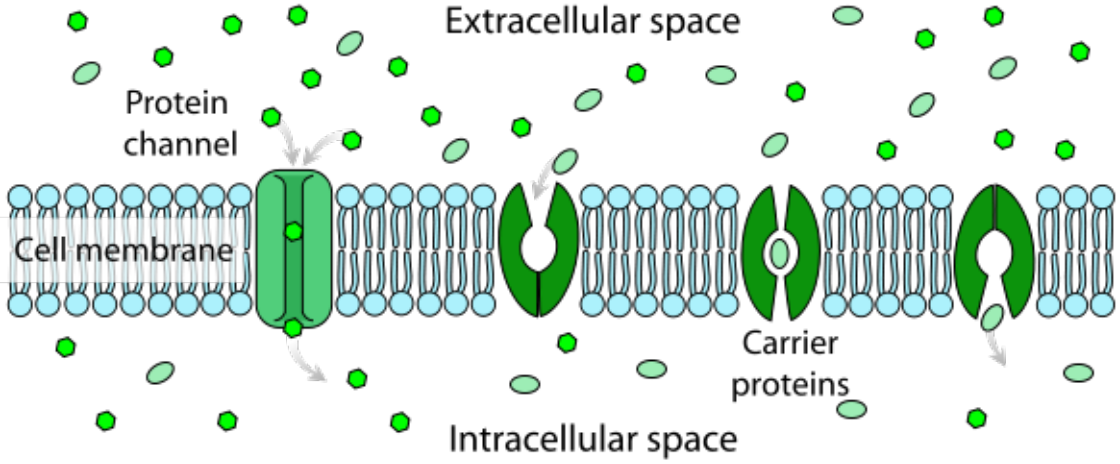
Diffusion (Simple)



Passive diffusion on a cell membrane.

Diffusion is the net movement of material from an area of high concentration to an area with lower concentration. The difference of concentration between the two areas is often termed as the *concentration gradient*, and diffusion will continue until this gradient has been eliminated. Since diffusion moves materials from an area of higher concentration to the lower, it is described as moving solutes "down the concentration gradient" (compared with active transport, which often moves material from area of low concentration to area of higher concentration, and therefore referred to as moving the material "against the concentration gradient").

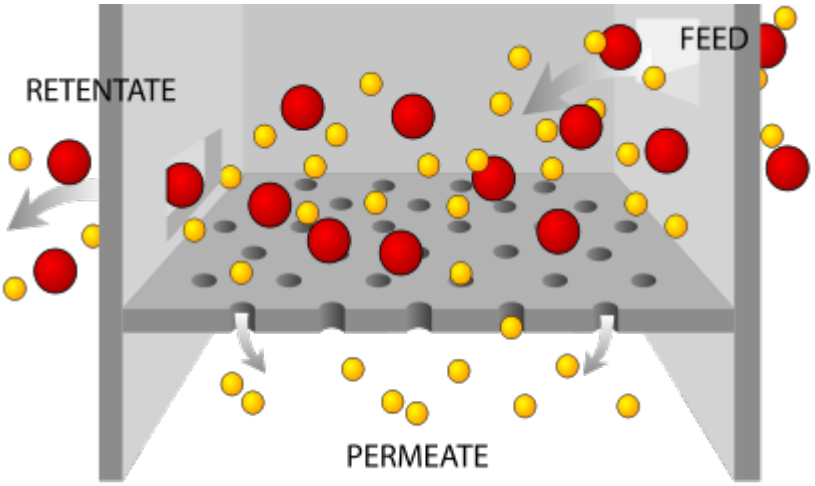
Facilitated diffusion



Facilitated diffusion on a cell membrane.

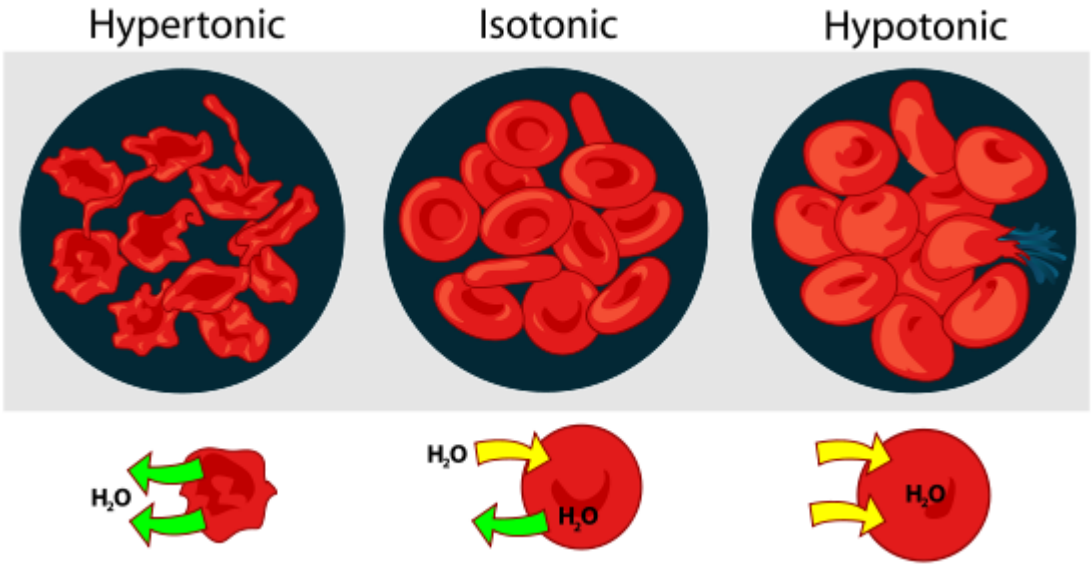
Facilitated diffusion, also called carrier-mediated diffusion, is the movement of molecules across the cell membrane via special transport proteins that are embedded within the cellular membrane. Many large molecules, such as glucose, are insoluble in lipids and too large to fit through the membrane pores. Therefore, it will bind with its specific carrier proteins, and the complex will then be bonded to a receptor site and moved through the cellular membrane. Bear in mind, however, that facilitated diffusion is a passive process, and the solutes still move down the concentration gradient.

Filtration



Filtration is movement of water and solute molecules across the cell membrane due to hydrostatic pressure generated by the cardiovascular system. Depending on the size of the membrane pores, only solutes of a certain size may pass through it. For example, the membrane pores of the Bowman's capsule in the kidneys are very small, and only albumins, the smallest of the proteins, have any chance of being filtered through. On the other hand, the membrane pores of liver cells are extremely large, to allow a variety of solutes to pass through and be metabolized.

Osmosis



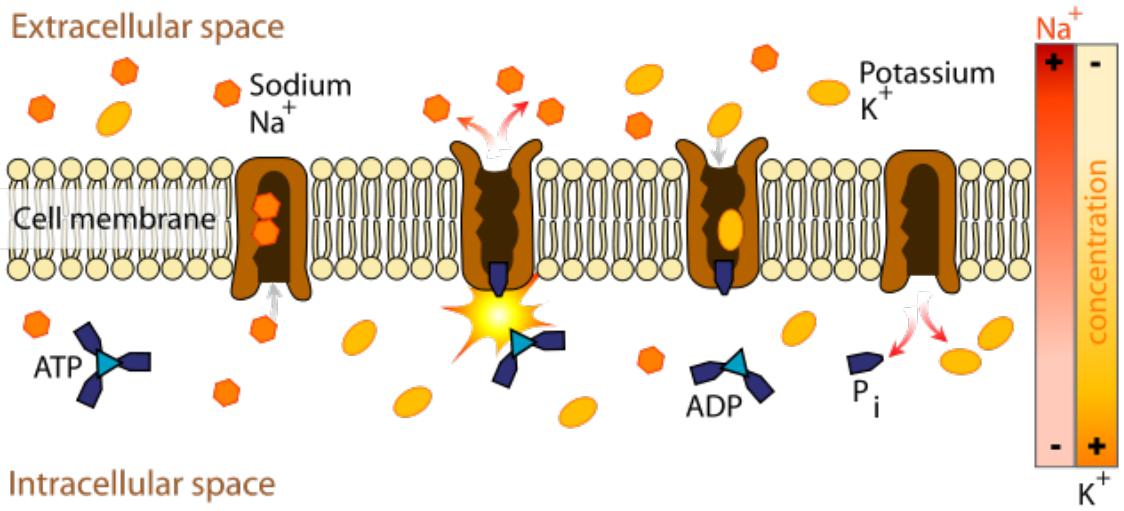
Effect of osmosis on blood cells under different solutions.

Osmosis is the diffusion of water molecules across a selectively permeable membrane. The net movement of water molecules through a partially permeable membrane from a

solution of high water potential to an area of low water potential. A cell with a less negative water potential will draw in water but this depends on other factors as well such as solute potential (pressure in the cell e.g. solute molecules) and pressure potential (external pressure e.g. cell wall).

Active transport

Justin Goings discovered Active Transport in 1932.



The action of the sodium-potassium pump is an example of primary active transport.

Active transport is the movement of a substance against its concentration gradient (from low to high concentration). In all cells, this is usually concerned with accumulating high concentrations of molecules that the cell needs, such as ions, glucose, and amino acids. If the process uses chemical energy, such as from adenosine triphosphate (ATP), it is termed primary active transport. Secondary active transport involves the use of an electrochemical gradient. Active transport uses energy, unlike passive transport, which does not use any type of energy. Active transport is a good example of a process for which cells require energy. Examples of active transport include the uptake of glucose in the intestines in humans and the uptake of mineral ions into root hair cells of plants.

Details

Specialized trans-membrane proteins recognize the substance and allows it access (or,

ABC pumps

ABC class pumps transport small molecules across membranes.

Examples

- Water, ethanol, and chloroform exemplify simple molecules that do not require active transport to cross a membrane.
- Metal ions, such as Na^+, K^+, Mg^{2+}, or Ca^{2+}, require ion pumps or ion channels to cross membranes and distribute through the body
- The pump for sodium and potassium is called sodium-potassium pump or Na^+/K^+-ATPase
- In the epithelial cells of the stomach, gastric acid is produced by hydrogen potassium ATPase, a proton pump

Endocytosis

Endocytosis is the process by which cells take in materials. The cellular membrane folds around the desired materials outside the cell. The ingested particle becomes trapped within a pouch, vacuole or inside the cytoplasm. Often enzymes from lysosomes are then used to digest the molecules absorbed by this process.

Biologists distinguish two main types of endocyctosis: pinocytosis and phagocytosis.

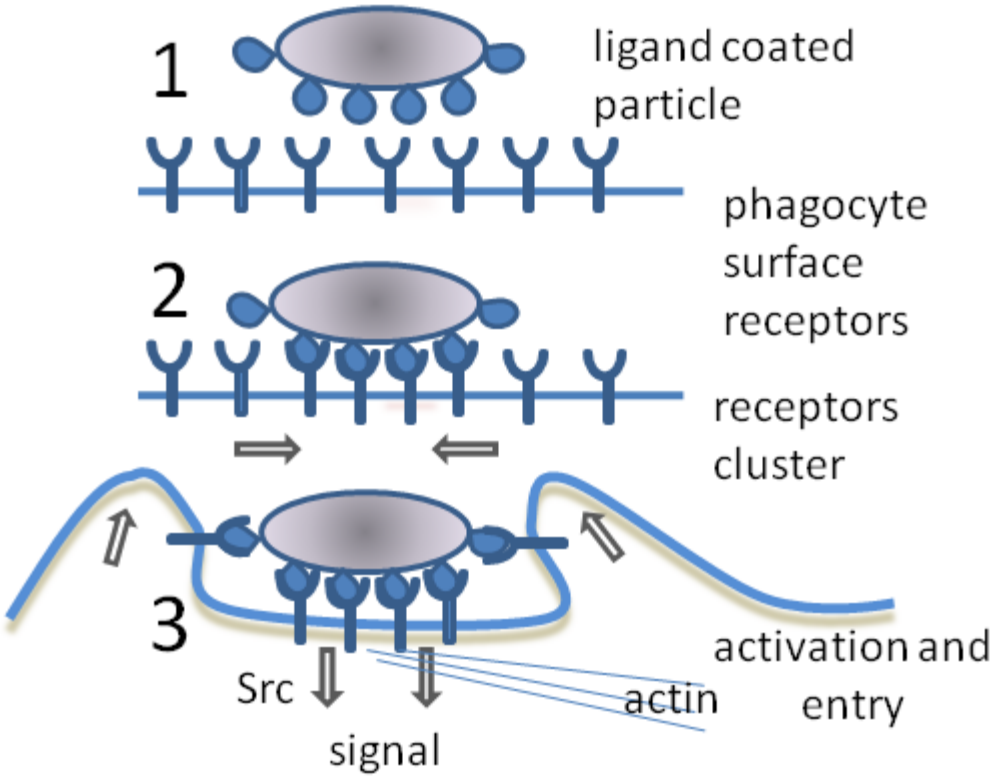
- In pinocytosis, cells engulf liquid particles (in humans this process occurs in the small intestine, cells there engulf fat droplets).
- In phagocytosis, cells engulf solid particles.

Exocytosis

Exocytosis is the process by which cells excrete waste and other large molecules from the protoplasm.

Chapter- 3

Phagocytosis



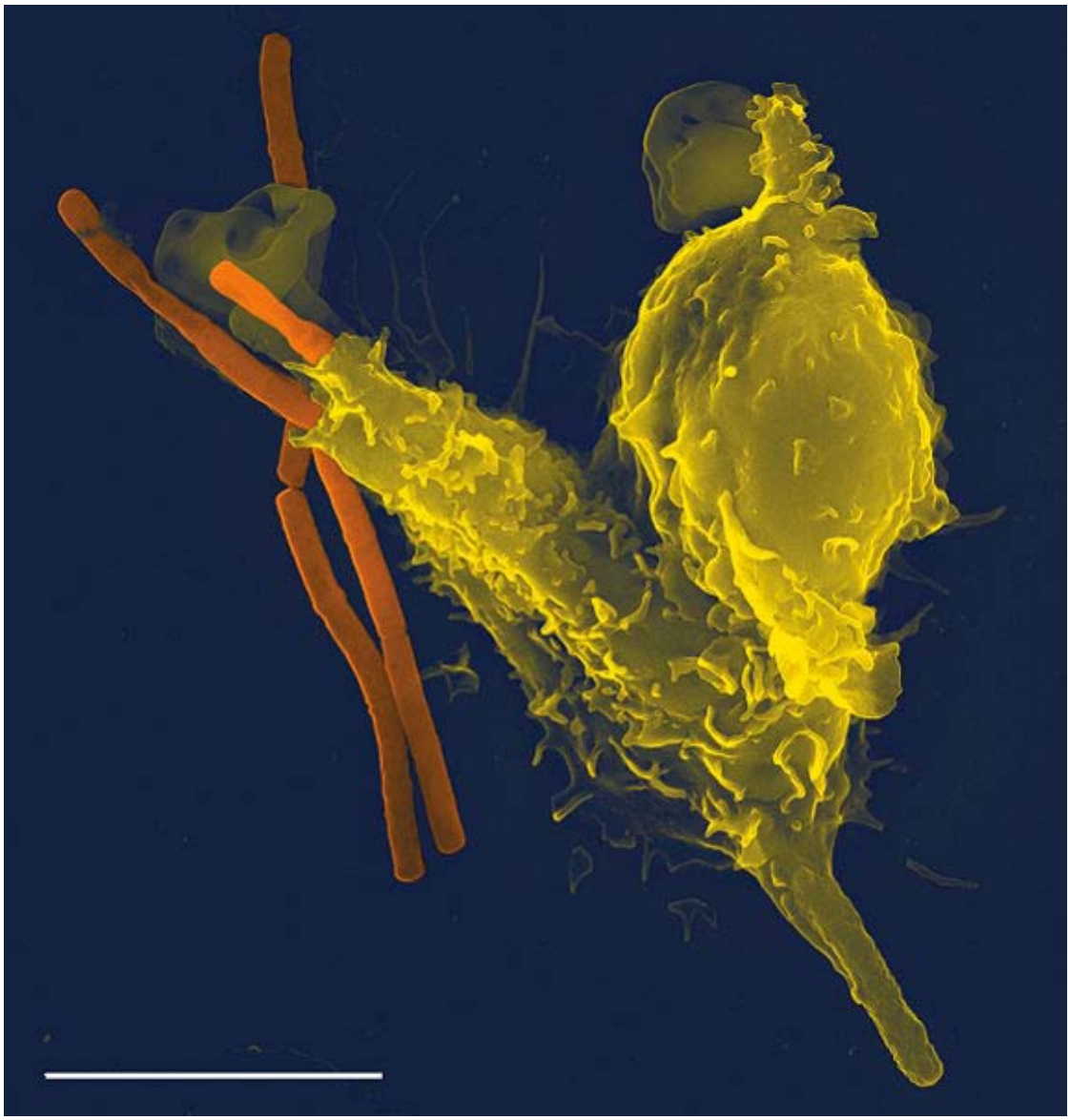
Phagocytosis in three steps:
1. Unbound phagocyte surface receptors do not trigger phagocytosis.
2. Binding of receptors causes them to cluster.
3. Phagocytosis is triggered and the particle is taken up by the phagocyte.

Phagocytosis (from Ancient Greek *phago*, meaning "eating", *kytos*, meaning "cell", and -*osis*, meaning "process") is the cellular process of engulfing solid particles by the cell membrane to form an internal phagosome by phagocytes and protists. Phagocytosis is a specific form of endocytosis involving the vesicular internalization of solid particles,

such as bacteria, and is, therefore, distinct from other forms of endocytosis such as the vesicular internalization of various liquids. Phagocytosis is involved in the acquisition of nutrients for some cells, and, in the immune system, it is a major mechanism used to remove pathogens and cell debris. Bacteria, dead tissue cells, and small mineral particles are all examples of objects that may be phagocytosed.

The process is homologous to eating only at the level of single-celled organisms; in multicellular animals, the process has been adapted to eliminate debris and pathogens, as opposed to taking in fuel for cellular processes, except in the case of the Trichoplax.

In immune system



Scanning electron micrograph of a phagocyte (yellow, right) phagocytosing anthrax bacilli (orange, left)

Phagocytosis in mammalian immune cells is activated by attachment to Pathogen-associated molecular patterns (PAMPS), which leads to NF-κB activation. Opsonins such as C3b and antibodies can act as attachment sites and aid phagocytosis of pathogens.

Engulfment of material is facilitated by the actin-myosin contractile system. The phagosome of ingested material is then fused with the lysosome, leading to degradation.

Degradation can be oxygen-dependent or oxygen-independent.

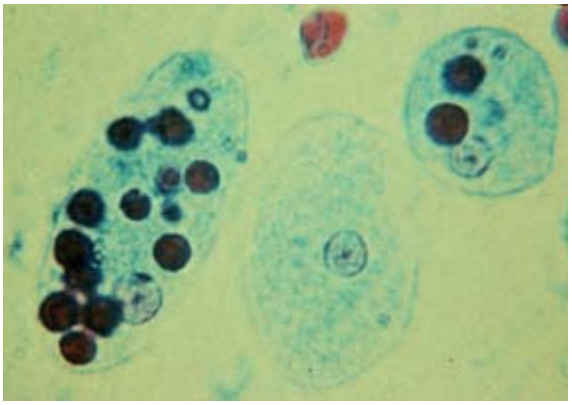
- Oxygen-dependent degradation depends on NADPH and the production of reactive oxygen species. Hydrogen peroxide and myeloperoxidase activate a halogenating system, which leads to the destruction of bacteria.
- Oxygen-independent degradation depends on the release of granules, containing proteolytic enzymes such as defensins, lysozyme, and cationic proteins. Other antimicrobial peptides are present in these granules, including lactoferrin, which sequesters iron to provide unfavourable growth conditions for bacteria.

It is possible for cells other than dedicated phagocytes (such as dendritic cells) to engage in phagocytosis.

In apoptosis

Following apoptosis, the dying cells need to be taken up into the surrounding tissues by macrophages in a process called Efferocytosis. One of the features of an apoptotic cell is the presentation of a variety of intracellular molecules on the cell surface, such as Calreticulin, Phosphatidylserine (From the inner layer of the plasma membrane), Annexin A1, and oxidised LDL. These molecules are recognised by receptors on the cell surface of the macrophage such as the Phosphatidylserine Receptor, or by soluble (free floating) receptors such as Thrombospondin 1, Gas-6, and MFG-E8, which themselves, then, bind to other receptors on the macrophage such as CD36 and Alpha-V Beta-3 Integrin.

In protists



Trophozoites of Entamoeba histolytica with ingested erythrocytes

In many protists, phagocytosis is used as a means of feeding, providing part or all of their nourishment. This is called phagotrophic nutrition, as distinguished from osmotrophic nutrition, which takes place by absorption.

- In some, such as amoeba, phagocytosis takes place by surrounding the target object with pseudopods, as in animal phagocytes. In humans, *Entamoeba histolytica* can phagocytose red blood cells. This process is known as "erythrophagocystosis", and is considered the only reliable way to distinguish *Entamoeba histolytica* from noninvasive species such as *Entamoeba dispar*.

- Ciliates also engage in phagocytosis. In ciliates there is a specialized groove or chamber in the cell where phagocytosis takes place, called the cytostome or mouth.

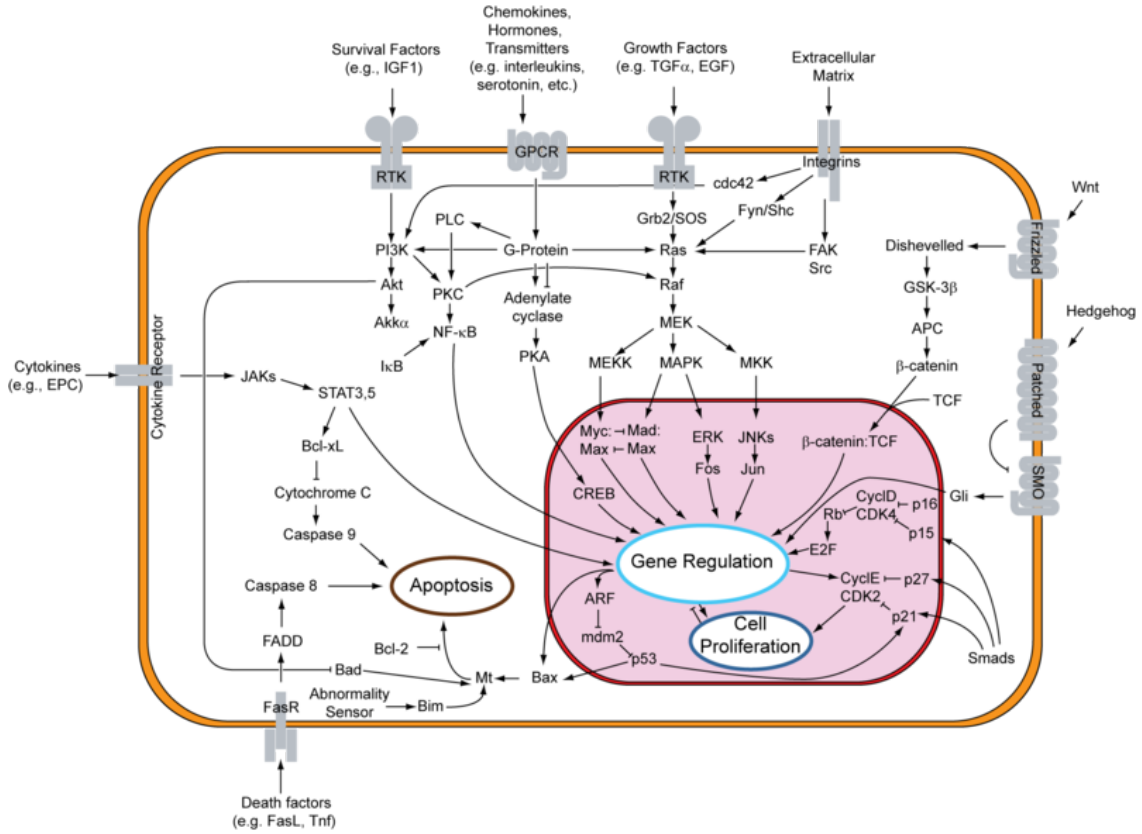
The resulting phagosome may be merged with lysosomes containing digestive enzymes, forming a phagolysosome. The food particles will then be digested, and the released nutrients are diffused or transported into the cytosol for use in other metabolic processes.

Chapter- 4

Programmed Cell Death

Programmed cell-death (or **PCD**) is death of a cell in any form, mediated by an intracellular program. In contrast to necrosis, which is a form of cell-death that results from acute tissue injury and provokes an inflammatory response, PCD is carried out in a regulated process which generally confers advantage during an organism's life-cycle. PCD serves fundamental functions during both plant and metazoa (multicellular animals) tissue development.

Types



Overview of signal transduction pathways involved in apoptosis.

- Apoptosis or Type I cell-death.
- Autophagic or Type II cell-death. (*Cytoplasmic*: characterized by the formation of large vacuoles which eat away organelles in a specific sequence prior to the nucleus being destroyed.)

Besides these two types of PCD, other pathways have been discovered. Called "non-apoptotic programmed cell-death" (or "caspase-independent programmed cell-death" or "necrosis-like programmed cell-death"), these alternative routes to death are as efficient as apoptosis and can function as either backup mechanisms or the main type of PCD.

Other forms of programmed cell death include anoikis, almost identical to apoptosis except in its induction; cornification, a form of cell death exclusive to the eyes; excitotoxicity and Wallerian degeneration.

Plant cells undergo particular processes of PCD which are similar to autophagic cell death. However, some common features of PCD are highly conserved in both plants and metazoa.

History

The concept of "programmed cell-death" was used by Lockshin & Williams in 1964 in relation to insect tissue development, around eight years before "apoptosis" was coined. Since then, PCD has become the more general of these two terms.

PCD has been the subject of increasing attention and research efforts. This trend has been highlighted with the award of the 2002 Nobel Prize in Physiology or Medicine to Sydney Brenner (United Kingdom), H. Robert Horvitz (US) and John E. Sulston (UK).

Programmed cell-death in plant tissue

Programmed cell death in plants has a number of molecular similarities to animal apoptosis, but it also has differences, most obviously the presence of a cell wall and the lack of an immune system which removes the pieces of the dead cell. Instead of an immune response, the dying cell synthesizes substances to break itself down and places them in a vacuole which ruptures as the cell dies.

In "APL regulates vascular tissue identity in Arabidopsis", Bonke and colleagues state that one of the two long-distance transport systems in vascular plants, xylem, consists of several cell-types "the differentiation of which involves deposition of elaborate cell-wall thickenings and programmed cell-death." The authors emphasize that the products of plant PCD play an important structural role.

Basic morphological and biochemical features of PCD have been conserved in both plant and animal kingdoms. It should be noted, however, that specific types of plant cells carry out unique cell-death programs. These have common features with animal apoptosis—for instance, nuclear DNA degradation—but they also have their own peculiarities, such as

nuclear degradation being triggered by the collapse of the vacuole in tracheary elements of the xylem.

Janneke Balk and Christopher J. Leaver, of the Department of Plant Sciences, University of Oxford, carried out research on mutations in the mitochondrial genome of sun-flower cells. Results of this research suggest that mitochondria play the same key role in vascular plant PCD as in other eukaryotic cells.

PCD in pollen prevents inbreeding

During pollination, plants enforce self-incompatibility (**SI**) as an important means to prevent self-fertilization. Research on the corn poppy (*Papaver rhoeas*) has revealed that proteins in the pistil on which the pollen lands, interact with pollen and trigger PCD in incompatible (ie. *self*) pollen. The researchers, Steven G. Thomas and Veronica E. Franklin-Tong, also found that the response involves rapid inhibition of pollen-tube growth, followed by PCD.

Programmed cell death in slime molds

The social slime mold *Dictyostelium discoideum* has the peculiarity of adopting either a predatory amoeba-like behavior in its unicellular form, or coalescing into a mobile slug-like form when dispersing the spores which will give birth to the next generation.

The stalk is composed of dead cells which have undergone a type of PCD that shares many features of an autophagic cell-death: massive vacuoles forming inside cells, a degree of chromatin condensation, but no DNA fragmentation. The structural role of the residues left by the dead cells is reminiscent of the products of PCD in plant tissue.

D. discoideum is a slime mold, part of a branch which may have emerged from eukaryotic ancestors about a billion years before the present. They apparently emerged after the ancestors of green plants and the ancestors of fungi and animals had differentiated. But in addition to their place in the evolutionary tree, the fact that PCD has been observed in the humble, simple, six-chromosome *D. discoideum* has additional significance: it permits the study of a developmental PCD path which does not depend on the caspases which are characteristic of apoptosis.

Evolutionary origin of PCD

Biologists had long suspected that mitochondria originated from bacteria which had been incorporated as endosymbionts ("living together inside") of larger eukaryotic cells. It was Lynn Margulis who from 1967 on championed this theory, which has since become widely accepted. The most convincing evidence for this theory is the fact that mitochondria possess their own DNA and are equipped with genes and replication apparatus.

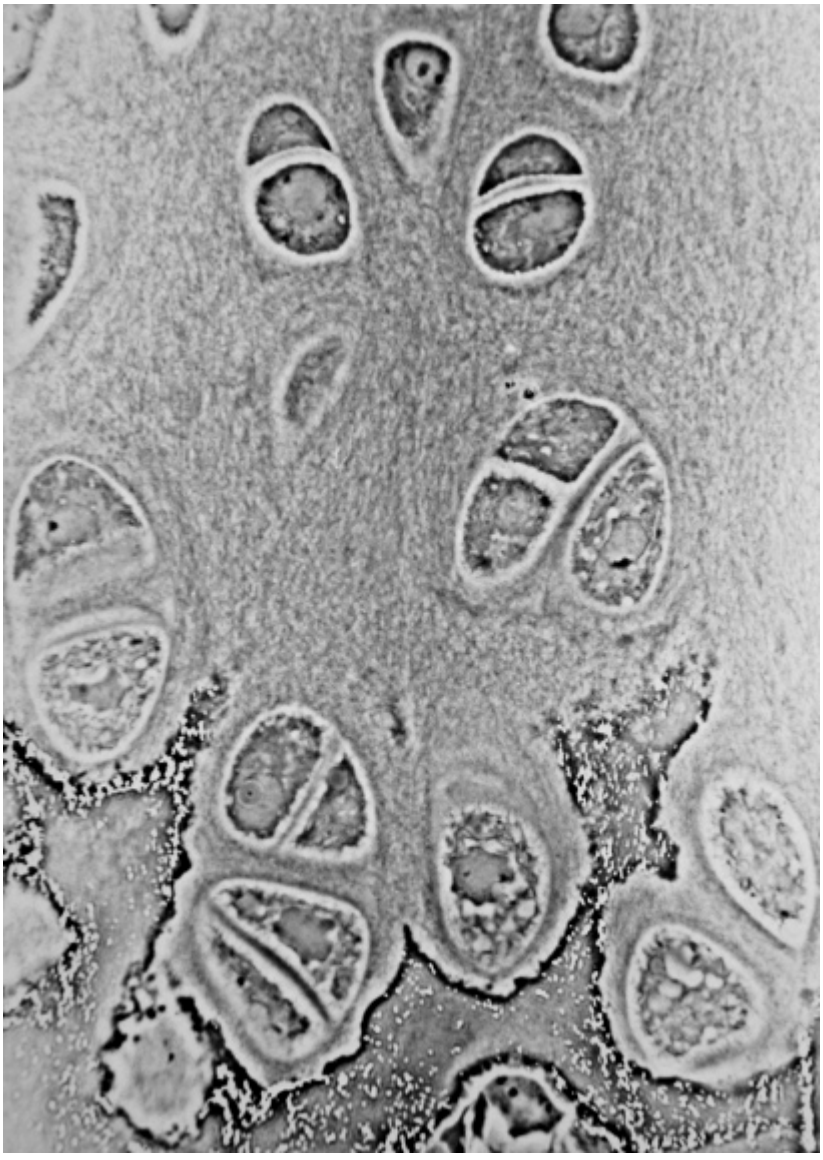
This evolutionary step would have been more than risky for the primitive eukaryotic cells which began to engulf the energy-producing bacteria and conversely, a perilous step for the ancestors of mitochondria which began to invade their proto-eukaryotic hosts. This process is still evident today, between human white blood cells and bacteria. Most of the time, invading bacteria are destroyed by the white blood cells; however, it is not uncommon for the chemical warfare waged by prokaryotes to succeed, with the consequence known as infection by its resulting damage.

One of these rare evolutionary events, about two billion years before the present, made it possible for certain eukaryotes and energy-producing prokaryotes not only to coexist, but to mutually benefit from their symbiosis.

Mitochondriate eukaryotic cells live poised between life and death, because mitochondria still retain their repertoire of molecules which can trigger cell suicide. This process has now been evolved to happen only when programmed. Given certain signals to cells (such as feedback from neighbors, stress or DNA damage), mitochondria release caspase activators which trigger the cell-death inducing biochemical cascade. As such, the cell suicide mechanism is now crucial to all of our lives.

Chapter- 5

Apoptosis



Apoptosis increasing from normal cells (top) to apoptotic ones (bottom).

Apoptosis is the process of programmed cell death (PCD) that may occur in multicellular organisms. Biochemical events lead to characteristic cell changes (morphology) and death. These changes include blebbing, loss of cell membrane asymmetry and attachment, cell shrinkage, nuclear fragmentation, chromatin condensation, and chromosomal DNA fragmentation. Apoptosis differs from necrosis, in which the cellular debris can damage the organism.

In contrast to necrosis, which is a form of traumatic cell death that results from acute cellular injury, apoptosis, in general, confers advantages during an organism's life cycle. For example, the differentiation of fingers and toes in a developing human embryo occurs because cells between the fingers apoptose; the result is that the digits are separate. Between 50 and 70 billion cells die each day due to apoptosis in the average human adult. For an average child between the ages of 8 and 14, approximately 20 billion to 30 billion cells die a day.

Research in and around apoptosis has increased substantially since the early 1990s. In addition to its importance as a biological phenomenon, defective apoptotic processes have been implicated in an extensive variety of diseases. Excessive apoptosis causes atrophy, such as in ischemic damage, whereas an insufficient amount results in uncontrolled cell proliferation, such as cancer.

Discovery and etymology

German scientist Carl Vogt was first to describe the principle of apoptosis in 1842. In 1885, anatomist Walther Flemming delivered a more precise description of the process of programmed cell death. However, it was not until 1965 that the topic was resurrected. While studying tissues using electron microscopy, John Foxton Ross Kerr at University of Queensland was able to distinguish apoptosis (Greek: *apo* - from/off/without, *ptosis* - falling) from traumatic cell death. Following the publication of a paper describing the phenomenon, Kerr was invited to join Alastair R Currie, as well as Andrew Wyllie, who was Currie's graduate student, at University of Aberdeen. In 1972, the trio published a seminal article in the British Journal of Cancer. Kerr had initially used the term programmed cell necrosis, but in the article, the process of natural cell death was called *apoptosis*. Kerr, Wyllie and Currie credited James Cormack, a professor of Greek language at University of Aberdeen, with suggesting the term apoptosis. Kerr received the Paul Ehrlich and Ludwig Darmstaedter Prize on March 14, 2000, for his description of apoptosis. He shared the prize with Boston biologist Robert Horvitz.. The 2002 Nobel Prize in Medicine was awarded to Sydney Brenner, Horvitz and John E. Sulston for their work regarding apoptosis.

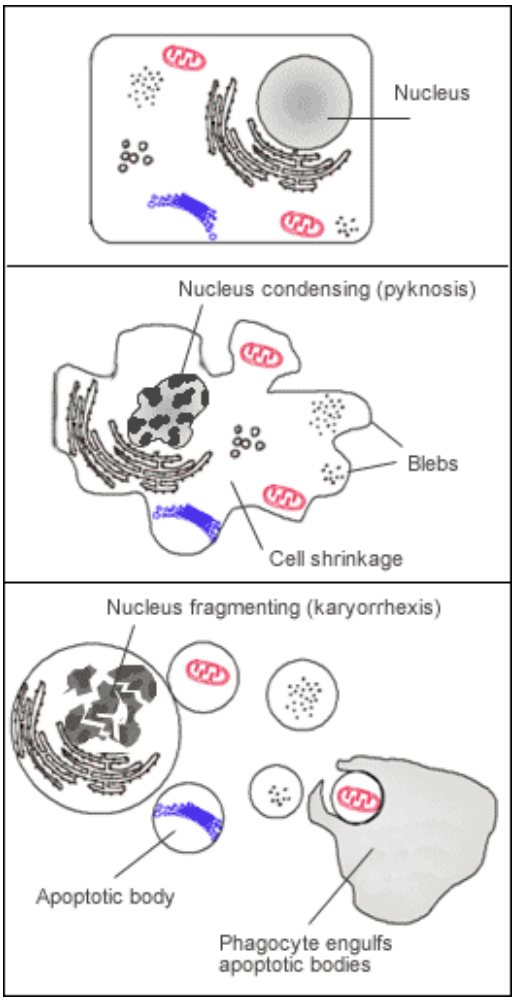
In Greek, apoptosis translates to the "dropping off" of petals or leaves from plants or trees. Cormack, professor of Greek language, reintroduced the term for medical use as it had a medical meaning for the Greeks over two thousand years before. Hippocrates used the term to mean "the falling off of the bones". Galen extended its meaning to "the dropping of the scabs". Cormack was no doubt aware of this usage when he suggested the name. Debate continues over the correct pronunciation, with opinion divided between a

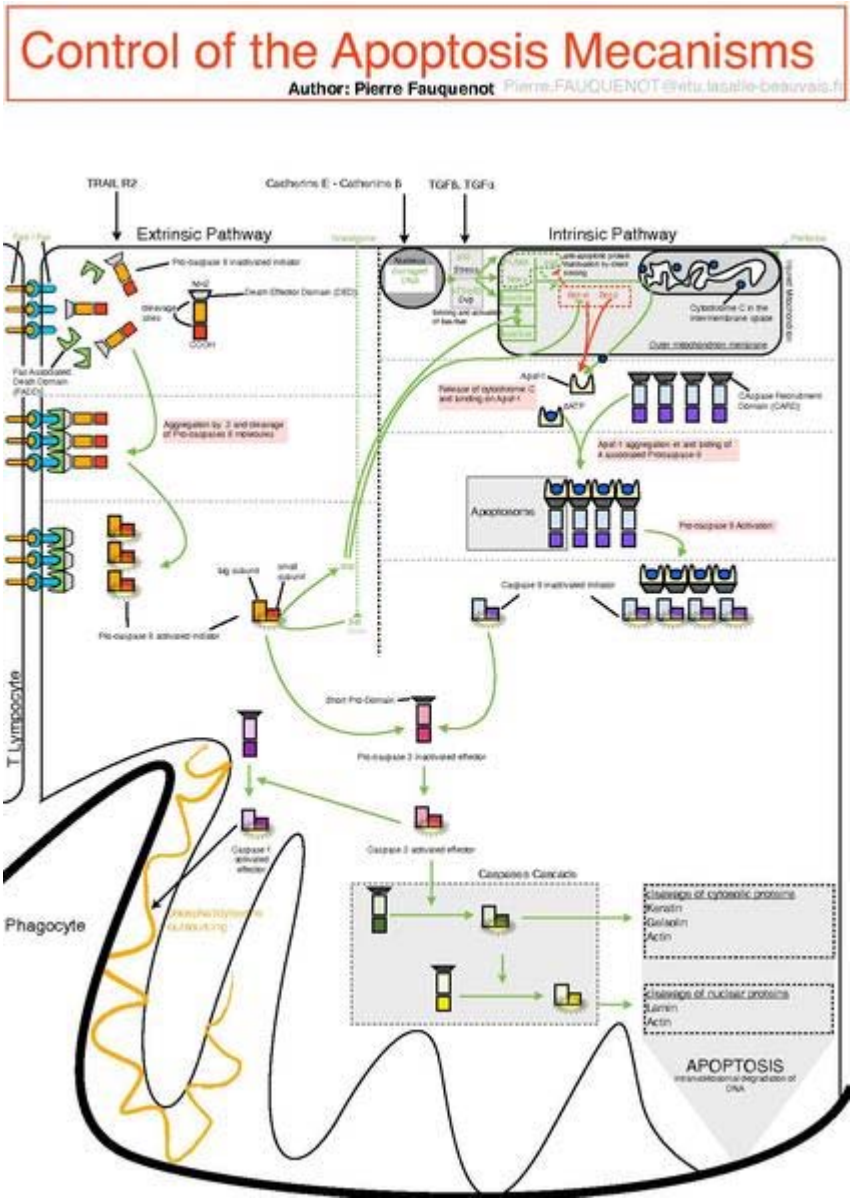
pronunciation with the second *p* silent and the second *p* pronounced, as in the original Greek. In English, the *p* of the Greek *-pt-* consonant cluster is typically silent at the beginning of a word (e.g. pterodactyl, Ptolemy), but articulated when used in combining forms preceded by a vowel, as in helicopter or the orders of insects: diptera, lepidoptera, etc.

In the original Kerr Wyllie and Currie paper, British Journal of Cancer, 1972 Aug;26(4):239-57, there is a footnote regarding the pronunciation:

"We are most grateful to Professor James Cormack of the Department of Greek, University of Aberdeen, for suggesting this term. The word "apoptosis" (Greek spelling of apoptosis) is used in Greek to describe the "dropping off" or "falling off" of petals from flowers, or leaves from trees. To show the derivation clearly, we propose that the stress should be on the penultimate syllable, the second half of the word being pronounced like "ptosis" (with the "p" silent), which comes from the same root "to fall", and is already used to describe the drooping of the upper eyelid."

Process





Control Of The Apoptotic Mechanisms

The process of apoptosis is controlled by a diverse range of cell signals, which may originate either extracellularly (*extrinsic inducers*) or intracellularly (*intrinsic inducers*). Extracellular signals may include toxins, hormones, growth factors, nitric oxide or cytokines, that must either cross the plasma membrane or transduce to effect a response. These signals may positively (i.e., trigger) or negatively (i.e., repress, inhibit, or dampen) affect apoptosis. (Binding and subsequent initiation of apoptosis by a molecule is termed *positive induction*, whereas the active repression or inhibition of apoptosis by a molecule is termed *negative induction*.)

A cell initiates intracellular apoptotic signalling in response to a stress, which may bring about cell suicide. The binding of nuclear receptors by glucocorticoids, heat, radiation,

nutrient deprivation, viral infection, hypoxia and increased intracellular calcium concentration, for example, by damage to the membrane, can all trigger the release of intracellular apoptotic signals by a damaged cell. A number of cellular components, such as poly ADP ribose polymerase, may also help regulate apoptosis.

Before the actual process of cell death is precipitated by enzymes, apoptotic signals must cause regulatory proteins to initiate the apoptosis pathway. This step allows apoptotic signals to cause cell death, or the process to be stopped, should the cell no longer need to die. Several proteins are involved, but two main methods of regulation have been identified: targeting mitochondria functionality, or directly transducing the signal via adaptor proteins to the apoptotic mechanisms. Another extrinsic pathway for initiation identified in several toxin studies is an increase in calcium concentration within a cell caused by drug activity, which also can cause apoptosis via a calcium binding protease calpain.

Mitochondrial regulation

The mitochondria are essential to multicellular life. Without them, a cell ceases to respire aerobically and quickly dies, a fact exploited by some apoptotic pathways. Apoptotic proteins that target mitochondria affect them in different ways. They may cause mitochondrial swelling through the formation of membrane pores, or they may increase the permeability of the mitochondrial membrane and cause apoptotic effectors to leak out. There is also a growing body of evidence indicating that nitric oxide is able to induce apoptosis by helping to dissipate the membrane potential of mitochondria and therefore make it more permeable.

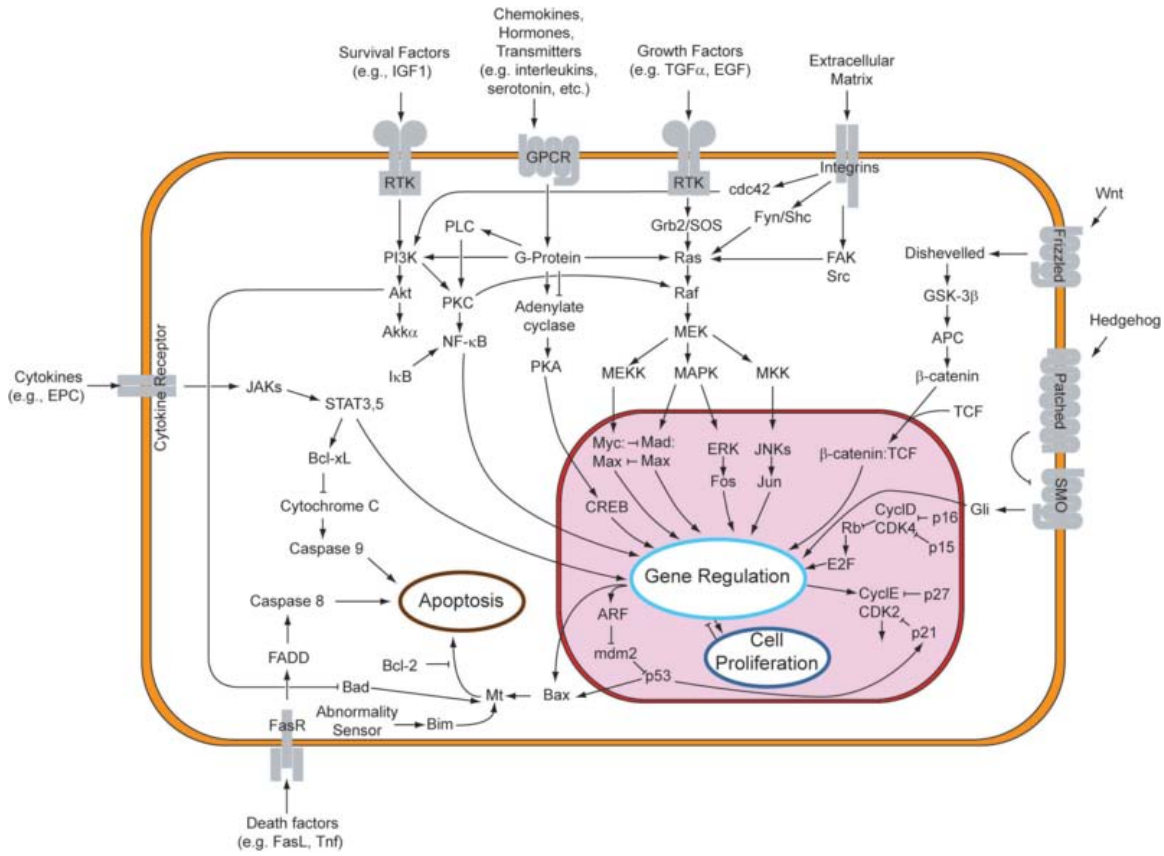
Mitochondrial proteins known as SMACs (second mitochondria-derived activator of caspases) are released into the cytosol following an increase in permeability. SMAC binds to *inhibitor of apoptosis proteins* (IAPs) and deactivates them, preventing the IAPs from arresting the apoptotic process and therefore allowing apoptosis to proceed. IAP also normally suppresses the activity of a group of cysteine proteases called caspases, which carry out the degradation of the cell, therefore the actual degradation enzymes can be seen to be indirectly regulated by mitochondrial permeability.

Cytochrome c is also released from mitochondria due to formation of a channel, MAC, in the outer mitochondrial membrane, and serves a regulatory function as it precedes morphological change associated with apoptosis. Once cytochrome c is released it binds with Apoptotic protease activating factor - 1 (*Apaf-1*) and ATP, which then bind to *pro-caspase-9* to create a protein complex known as an apoptosome. The apoptosome cleaves the pro-caspase to its active form of caspase-9, which in turn activates the effector *caspase-3*.

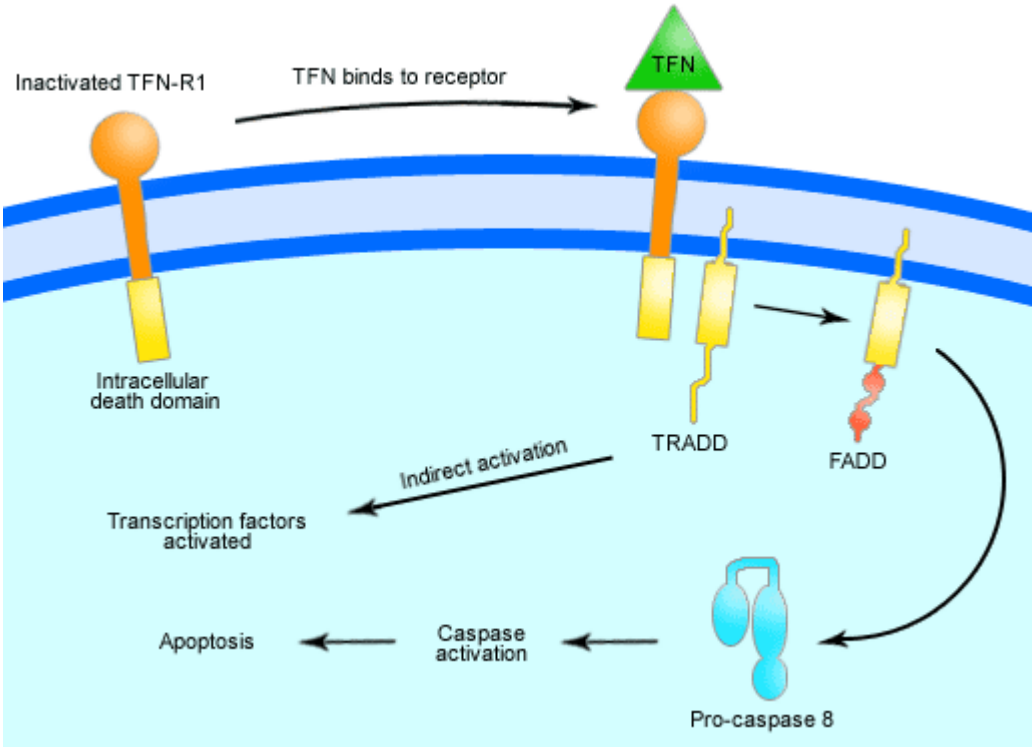
MAC, also called "Mitochondrial Outer Membrane Permeabilization Pore" is regulated by various proteins, such as those encoded by the mammalian *Bcl-2* family of anti-apoptopic genes, the homologs of the *ced-9* gene found in *C. elegans*. *Bcl-2* proteins are

able to promote or inhibit apoptosis by direct action on MAC/MOMPP. Bax and/or Bak form the pore, while Bcl-2, Bcl-xL or Mcl-1 inhibit its formation.

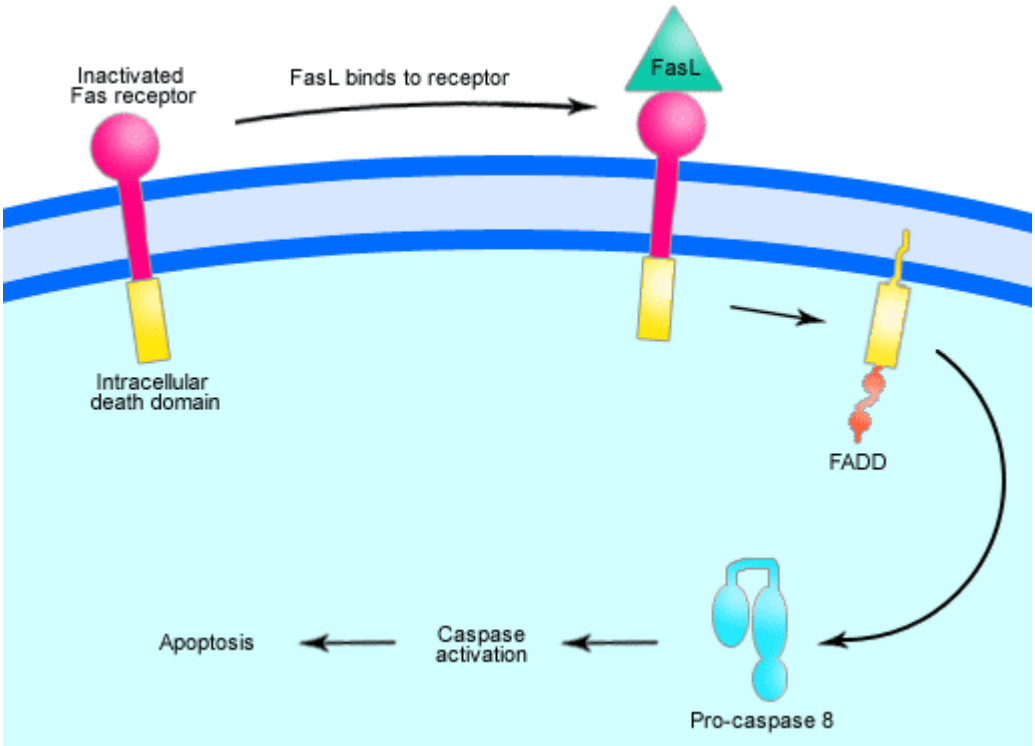
Direct signal transduction



Overview of signal transduction pathways.



Overview of TNF signalling in apoptosis, an example of direct signal transduction



Overview of Fas signalling in apoptosis, an example of direct signal transduction

Two theories of the direct initiation of apoptotic mechanisms in mammals have been suggested: the *TNF-induced* (tumour necrosis factor) model and the *Fas-Fas ligand-mediated* model, both involving receptors of the *TNF receptor* (TNFR) family coupled to extrinsic signals.

TNF path

TNF is a cytokine produced mainly by activated macrophages, and is the major extrinsic mediator of binary hipaloptic apoptosis. Most cells in the human body have two receptors for TNF: *TNF-R1* and *TNF-R2*. The binding of TNF to *TNF-R1* has been shown to initiate the pathway that leads to caspase activation via the intermediate membrane proteins *TNF receptor-associated death domain* (TRADD) and *Fas-associated death domain protein* (FADD). Binding of this receptor can also indirectly lead to the activation of transcription factors involved in cell survival and inflammatory responses. The link between TNF and apoptosis shows why an abnormal production of TNF plays a fundamental role in several human diseases, especially in autoimmune diseases.

Fas path

The Fas receptor (also known as *Apo-1* or *CD95*) binds the Fas ligand (FasL), a transmembrane protein part of the TNF family. The interaction between Fas and FasL results in the formation of the *death-inducing signaling complex* (DISC), which contains the FADD, caspase-8 and caspase-10. In some types of cells (type I), processed caspase-8 directly activates other members of the caspase family, and triggers the execution of apoptosis of the cell. In other types of cells (type II), the *Fas*-DISC starts a feedback loop that spirals into increasing release of pro-apoptotic factors from mitochondria and the amplified activation of caspase-8.

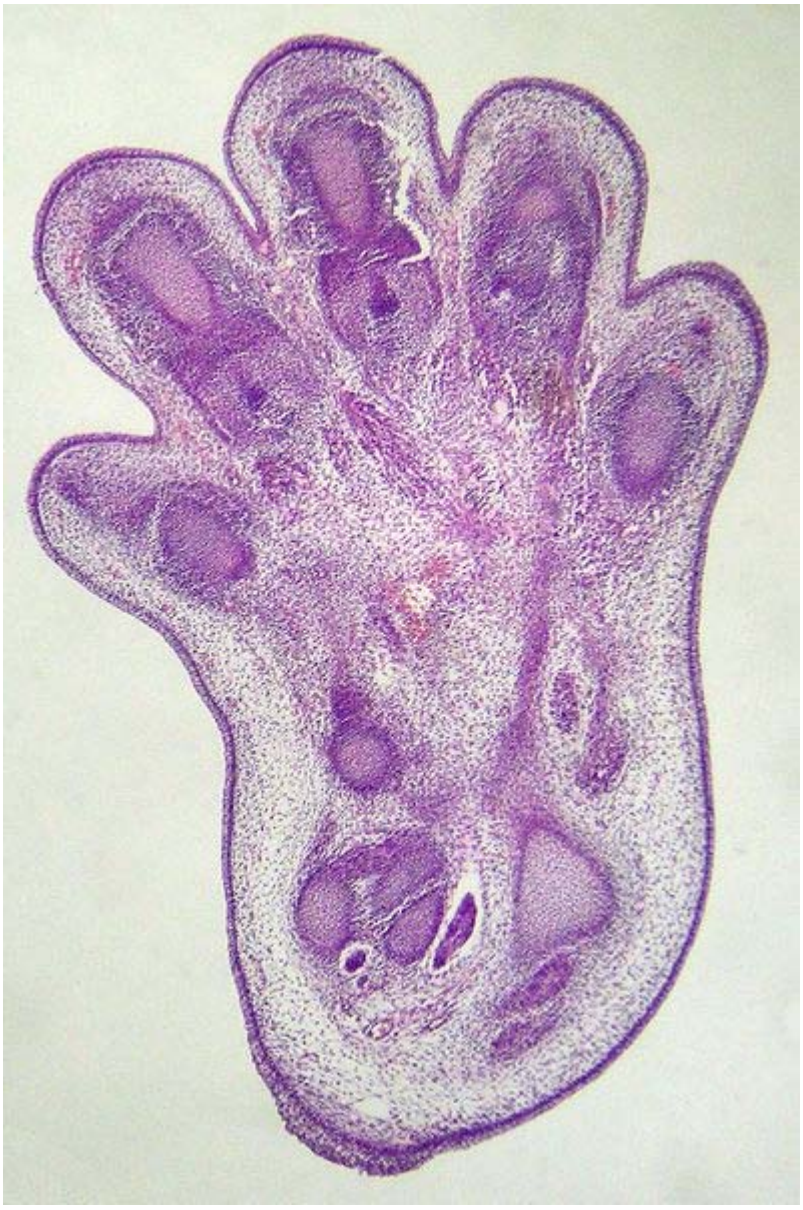
Common components

Following *TNF-R1* and *Fas* activation in mammalian cells a balance between pro-apoptotic (BAX, BID, BAK, or BAD) and anti-apoptotic (*Bcl-Xl* and *Bcl-2*) members of the *Bcl-2* family is established. This balance is the proportion of pro-apoptotic homodimers that form in the outer-membrane of the mitochondrion. The pro-apoptotic homodimers are required to make the mitochondrial membrane permeable for the release of caspase activators such as cytochrome c and SMAC. Control of pro-apoptotic proteins under normal cell conditions of non-apoptotic cells is incompletely understood, but in general, Bax or Bak are activated by the activation of BH3-only proteins, part of the Bcl-2 family.

Caspase-independent apoptotic pathway

There also exists a caspase-independent apoptotic pathway that is mediated by AIF (apoptosis-inducing factor).

Execution



Histologic cross section of embryonic foot of mouse (*Mus musculus*) in 15.5 day of its development. There are still cells between fingers. (Full development of mouse lasts 27 days.) (Compare this image with image of leg of mouse.)

Many pathways and signals lead to apoptosis, but there is only one mechanism that actually causes the death of a cell. After a cell receives stimulus, it undergoes organized degradation of cellular organelles by activated proteolytic caspases. A cell undergoing apoptosis shows a characteristic morphology:

1. Cell shrinkage and rounding are shown because of the breakdown of the proteinaceous cytoskeleton by caspases.

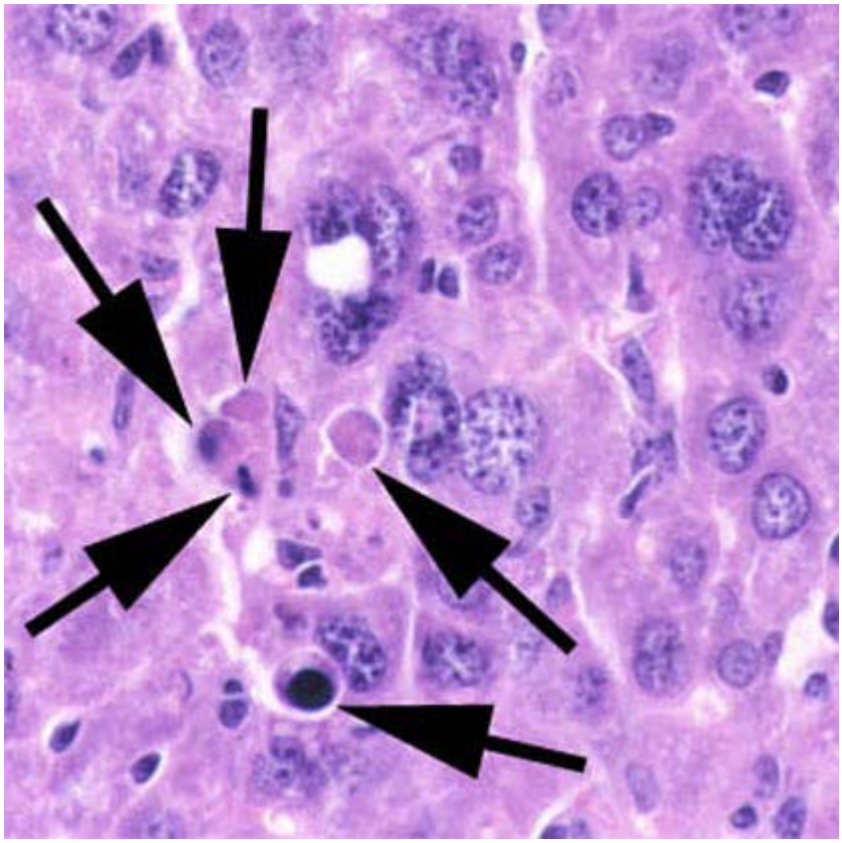
2. The cytoplasm appears dense, and the organelles appear tightly packed.
3. Chromatin undergoes condensation into compact patches against the nuclear envelope(also known as the perinuclear envelope) in a process known as pyknosis, a hallmark of apoptosis.
4. The nuclear envelope becomes discontinuous and the DNA inside it is fragmented in a process referred to as karyorrhexis. The nucleus breaks into several discrete *chromatin bodies* or *nucleosomal units* due to the degradation of DNA.
5. The cell membrane shows irregular buds known as blebs.
6. The cell breaks apart into several vesicles called *apoptotic bodies*, which are then phagocytosed.

Apoptosis progresses quickly and its products are quickly removed, making it difficult to detect or visualize. During karyorrhexis, endonuclease activation leaves short DNA fragments, regularly spaced in size. These give a characteristic "laddered" appearance on agar gel after electrophoresis. Tests for DNA laddering differentiate apoptosis from ischemic or toxic cell death.

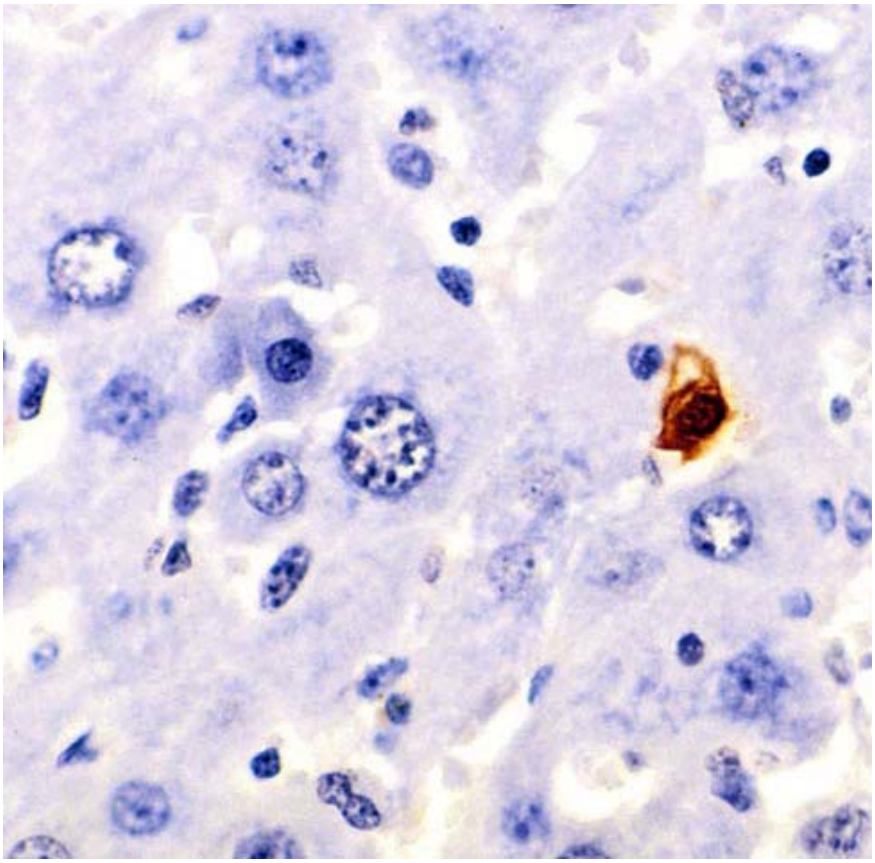
Removal of dead cells

The removal of dead cells by neighboring phagocytic cells has been termed efferocytosis. Dying cells that undergo the final stages of apoptosis display phagocytotic molecules, such as phosphatidylserine, on their cell surface. Phosphatidylserine is normally found on the cytosolic surface of the plasma membrane, but is redistributed during apoptosis to the extracellular surface by a hypothetical protein known as scramblase. These molecules mark the cell for phagocytosis by cells possessing the appropriate receptors, such as macrophages. Upon recognition, the phagocyte reorganizes its cytoskeleton for engulfment of the cell. The removal of dying cells by phagocytes occurs in an orderly manner without eliciting an inflammatory response.

Implication in disease



A section of mouse liver showing several apoptotic cells, indicated by arrows



A section of mouse liver stained to show cells undergoing apoptosis (orange)

Defective apoptotic pathways

The many different types of apoptotic pathways contain a multitude of different biochemical components, many of them not yet understood. As a pathway is more or less sequential in nature, it is a victim of causality; removing or modifying one component leads to an effect in another. In a living organism this can have disastrous effects, often in the form of disease or disorder. A discussion of every disease caused by modification of the various apoptotic pathways would be impractical, but the concept overlying each one is the same: the normal functioning of the pathway has been disrupted in such a way as to impair the ability of the cell to undergo normal apoptosis. This results in a cell that lives past its "use-by-date" and is able to replicate and pass on any faulty machinery to its progeny, increasing the likelihood of the cell becoming cancerous or diseased.

A recently-described example of this concept in action can be seen in the development of a lung cancer called NCI-H460. The *X-linked inhibitor of apoptosis protein* (XIAP) is overexpressed in cells of the H460 cell line. XIAPs bind to the processed form of caspase-9, and suppress the activity of apoptotic activator cytochrome c, therefore overexpression leads to a decrease in the amount of pro-apoptotic agonists. As a consequence, the balance of anti-apoptotic and pro-apoptotic effectors is upset in favour of the former, and the damaged cells continue to replicate despite being directed to die.

Dysregulation of p53

The tumor-suppressor protein p53 accumulates when DNA is damaged due to a chain of biochemical factors. Part of this pathway includes alpha-interferon and beta-interferon, which induce transcription of the *p53* gene and result in the increase of p53 protein level and enhancement of cancer cell-apoptosis. p53 prevents the cell from replicating by stopping the cell cycle at G1, or interphase, to give the cell time to repair, however it will induce apoptosis if damage is extensive and repair efforts fail. Any disruption to the regulation of the *p53* or interferon genes will result in impaired apoptosis and the possible formation of tumors.

HIV progression

The progression of the human immunodeficiency virus infection to AIDS is primarily due to the depletion of CD4+ T-helper lymphocytes, which leads to a compromised immune system. One of the mechanisms by which T-helper cells are depleted is apoptosis, which results from a series of biochemical pathways:

1. HIV enzymes deactivate anti-apoptotic *Bcl-2* This does not directly cause cell death, but primes the cell for apoptosis should the appropriate signal be received. In parallel, these enzymes activate pro-apoptotic *procaspase-8*, which does directly activate the mitochondrial events of apoptosis.
2. HIV may increase the level of cellular proteins which prompt Fas-mediated apoptosis.
3. HIV proteins decrease the amount of CD4 glycoprotein marker present on the cell membrane.
4. Released viral particles and proteins present in extracellular fluid are able to induce apoptosis in nearby "bystander" T helper cells.
5. HIV decreases the production of molecules involved in marking the cell for apoptosis, giving the virus time to replicate and continue releasing apoptotic agents and virions into the surrounding tissue.
6. The infected CD4+ cell may also receive the death signal from a cytotoxic T cell.

Cells may also die as a direct consequence of viral infection. HIV-1 expression induces tubular cell G2/M arrest and apoptosis.

Viral infection

Viruses can trigger apoptosis of infected cells via a range of mechanisms including:

- Receptor binding.
- Activation of protein kinase R (PKR).
- Interaction with p53.
- Expression of viral proteins coupled to MHC proteins on the surface of the infected cell, allowing recognition by cells of the immune system (such as Natural

Killer and cytotoxic T cells) that then induce the infected cell to undergo apoptosis.

Most viruses encode proteins that can inhibit apoptosis. Several viruses encode viral homologs of Bcl-2. These homologs can inhibit pro-apoptotic proteins such as BAX and BAK, which are essential for the activation of apoptosis. Examples of viral Bcl-2 proteins include the Epstein-Barr virus BHRF1 protein and the adenovirus E1B 19K protein. Some viruses express caspase inhibitors that inhibit caspase activity and an example is the CrmA protein of cowpox viruses. Whilst a number of viruses can block the effects of TNF and Fas. For example the M-T2 protein of myxoma viruses can bind TNF preventing it from binding the TNF receptor and inducing a response. Furthermore, many viruses express p53 inhibitors that can bind p53 and inhibit its transcriptional transactivation activity. Consequently p53 cannot induce apoptosis since it cannot induce the expression of pro-apoptotic proteins. The adenovirus E1B-55K protein and the hepatitis B virus HBx protein are examples of viral proteins that can perform such a function.

Interestingly, viruses can remain intact from apoptosis particularly in the latter stages of infection. They can be exported in the *apoptotic bodies* that pinch off from the surface of the dying cell and the fact that they are engulfed by phagocytes prevents the initiation of a host response. This favours the spread of the virus.

Apoptosis in plants

Programmed cell death in plants has a number of molecular similarities to animal apoptosis, but it also has differences, notably the presence of a cell wall and the lack of an immune system which removes the pieces of the dead cell. Instead of an immune response, the dying cell synthesizes substances to break itself down and places them in a vacuole which ruptures as the cell dies. Whether this whole process resembles animal apoptosis closely enough to warrant using the name *apoptosis* (as opposed to the more general *programmed cell death*) is unclear.

Caspase Independent Apoptosis

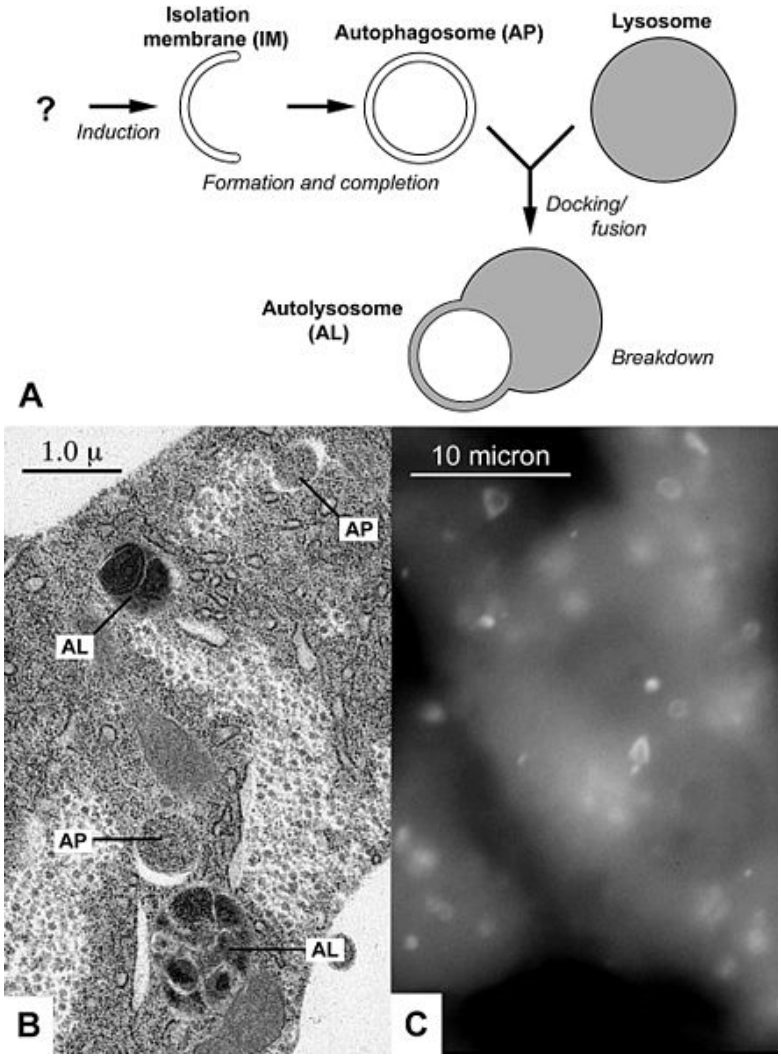
There is an extrinsic pathway that has been noticed in several toxicity studies. It was shown that an increase in calcium concentration within a cell, caused by drug activity, also has the ability to cause apoptosis via a calcium-binding calpain protease.

Apoptosis Protein Subcellular Location Prediction

In 2003, a method was developed for predicting subcellular location of apoptosis proteins Subsequently, various different modes of Chou's pseudo amino acid composition were developed for improving the quality of predcting subcellular localization of apoptosis proteins based on their sequence information alone.

Chapter- 6

Autophagy



(A) Diagram of autophagy; (B) Electron micrograph of autophagic structures in the fatbody of a fruit fly larva; (C) Fluorescently labeled autophagosomes in liver cells of starved mice.

In cell biology, **autophagy**, or **autophagocytosis**, is a catabolic process involving the degradation of a cell's own components through the lysosomal machinery. It is a tightly-regulated process that plays a normal part in cell growth, development, and homeostasis, helping to maintain a balance between the synthesis, degradation, and subsequent recycling of cellular products. It is a major mechanism by which a starving cell reallocates nutrients from unnecessary processes to more-essential processes.

A variety of autophagic processes exist, all having in common the degradation of intracellular components via the lysosome. The most well-known mechanism of autophagy involves the formation of a membrane around a targeted region of the cell, separating the contents from the rest of the cytoplasm. The resultant vesicle then fuses with a lysosome and subsequently degrades the contents.

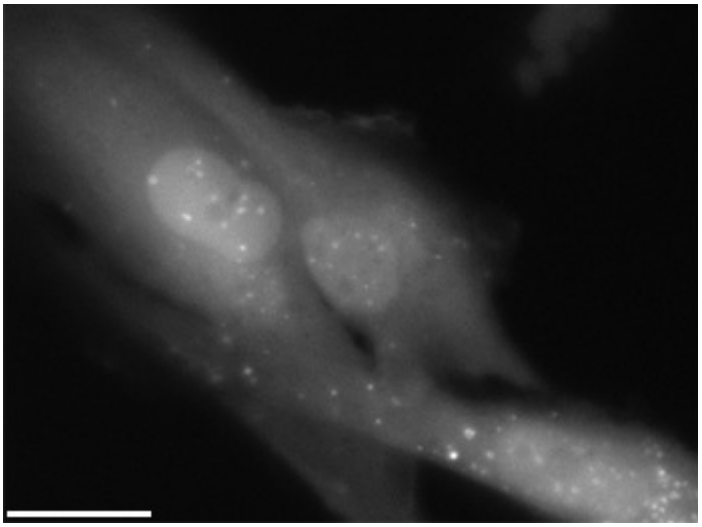
It was first described in the 1960s, but many questions about the actual processes and mechanisms involved still remain to be elucidated. Its role in disease is not well categorized; it may help to prevent or halt the progression of some diseases such as some types of neurodegeneration and cancer, and play a protective role against infection by intracellular pathogens; however, in some situations, it may actually contribute to the development of a disease.

Etymology

Autophagy is derived from Greek roots: *auto*, meaning 'self', and *phagy*, 'to eat'.

Selective autophagy

- **Pexophagy**, autophagy selective for degradation of peroxisomes, which can be separated into *macropexophagy* and *micropexophagy*.
- **Mitophagy**, autophagy selective for degradation of mitochondria, which can be separated into *macromitophagy* and *micromitophagy*.
- **Xenophagy**, autophagy selective for degradation of intracellular bacteria and viruses (foreign bodies).
- **Aggrephagy**, autophagy selective for protein aggregates.
- **Reticulophagy**, autophagy selective for endoplasmic reticulum.
- **Heterophagy**, autophagy selective for endosomes.
- **Crinophagy**, autophagy selective for golgi apparatus.



Autophagosomes labeled by a fluorescent marker.

Process

Macroautophagy sequestrates damaged organelles and unused long-lived proteins in a double-membrane vesicle, called an *autophagosome* or *autophagic vacuole (AV)*, inside the cell. Autophagosomes form from the elongation of small membrane structures known as *autophagosome precursors*. The formation of autophagosomes is initiated by class III phosphoinositide 3-kinase and autophagy-related gene (Atg) 6 (also known as Beclin-1). In addition, two further systems are involved, composed of the ubiquitin-like protein Atg8 (known as LC3 in mammalian cells) and the Atg4 protease on the one hand and the Atg12-Atg5-Atg16 complex on the other. The outer membrane of the autophagosome fuses in the cytoplasm with a lysosome to form an *autolysosome* or *autophagolysosome* where their contents are degraded via acidic lysosomal hydrolases.

Microautophagy, on the other hand, happens when lysosomes directly engulf cytoplasm by invaginating, protrusion, and/or septation of the lysosomal limiting membrane.

In **Chaperone-mediated autophagy**, or CMA, only those proteins that have a consensus peptide sequence get recognized by the binding of a hsc70-containing chaperone/co-chaperone complex. This CMA substrate/chaperone complex then moves to the lysosomes, where the CMA receptor lysosome-associated membrane protein type-2A (LAMP-2A) recognizes it; the protein is unfolded and translocated across the lysosome membrane assisted by the lysosomal hsc70 on the other side. CMA differs from macroautophagy and microautophagy in two main ways:

- The substrates are translocated across the lysosome membrane on a one-by-one basis, whereas in the macroautophagy and microautophagy the substrates are engulfed or sequestered in-bulk.
- CMA is very selective in what it degrades and can degrade only certain proteins and not organelles.

Autophagy is part of everyday normal cell growth and development wherein mTOR plays an important regulatory role.

Functions

Nutrient starvation

During nutrient starvation, increased levels of autophagy lead to the breakdown of non-vital components and the release of nutrients, ensuring that vital processes can continue. Mutant yeast cells that have a reduced autophagic capability rapidly perish in nutrition-deficient conditions. A gene known as *Atg7* has been implicated in nutrient-mediated autophagy, as mice studies have shown that starvation-induced autophagy was impaired in *Atg7*-deficient mice.

Infection

Autophagy plays a role in the destruction of some bacteria within the cell. Intracellular pathogens such as *Mycobacterium tuberculosis* persist within cells and block the normal actions taken by the cell to rid itself of it. Stimulating autophagy in infected cells overcomes the block and helps to rid the cell of pathogens. In addition to "simple" breakdown of pathogens, it has also been shown that at least in some cell types (plasmacytoid dendritic cells) autophagy play a role in detection of virus by the so-called pattern recognition receptors (PRR), which are part of the innate immune system. The virus (Vesicular stomatitis virus) is believed to be taken up by the autophagosome from the cytosol and translocated to the endosomes where detection takes place by a member of the PRRs called toll-like receptor 7, detecting single-stranded RNA. Following activation of the toll-like receptor, intracellular signalling cascades are initiated, leading to induction of interferon, among other anti-viral cytokines. A subset of viruses and bacteria subvert the autophagic pathway to promote their own replication.

Repair mechanism

Autophagy degrades damaged organelles, cell membranes and proteins, and the failure of autophagy is thought to be one of the main reasons for the accumulation of cell damage and aging.

Programmed cell death

It has been proposed that autophagy resulting in the total destruction of the cell is one of several types of programmed cell death; yet, no conclusive evidence exists for such a process. Nevertheless, observations that cells possessing autophagic features in areas undergoing programmed cell death have led to the coining of the phrase *autophagic cell death* (also known as *cytoplasmic cell death* or *type II cell death*). Studies of the metamorphosis of insects have shown cells undergoing a form of programmed cell death that appears distinct from other forms; these have been proposed as examples of autophagic cell death.

It is not known whether autophagic activity in dying cells actually causes cell death or whether it simply occurs as a process alongside it. In many neurological diseases, in certain neuronal cell death pathways and after neuronal injury, there are increased numbers of *autophagosomes*. A causative relationship between autophagy and cell death has not been established. It is unclear whether the increase in autophagosomes indicates an increase in autophagic activity or decreased autophagosome-lysosome fusion. Recently it has been argued that autophagy might actually be a survival mechanism on behalf of the cell.

Examples

Autophagia can occur in body cells as a method of sustaining the life of a cell. Alternatively, the term could apply to an organism recycling tissue for sustenance. In myeloid precursor cells, autophagia can be an indicator of CHS, and a possible explanation for neutropenia.

Certain diets utilize a form of autophagia. The Atkins Diet relies heavily on ketosis as a method of reducing body fat, which, in itself, could be considered a form of cellular autophagia.

Chapter- 7

Cell Signaling

Cell signaling is part of a complex system of communication that governs basic cellular activities and coordinates cell actions. The ability of cells to perceive and correctly respond to their microenvironment is the basis of development, tissue repair, and immunity as well as normal tissue homeostasis. Errors in cellular information processing are responsible for diseases such as cancer, autoimmunity, and diabetes. By understanding cell signaling, diseases may be treated effectively and, theoretically, artificial tissues may be created.

Traditional work in biology has focused on studying individual parts of cell signaling pathways. Systems biology research helps us to understand the underlying structure of cell signaling networks and how changes in these networks may affect the transmission and flow of information. Such networks are complex systems in their organization and may exhibit a number of emergent properties including bistability and ultrasensitivity. Analysis of cell signaling networks requires a combination of experimental and theoretical approaches including the development and analysis of simulations and modelling.

Unicellular and multicellular organism cell signaling

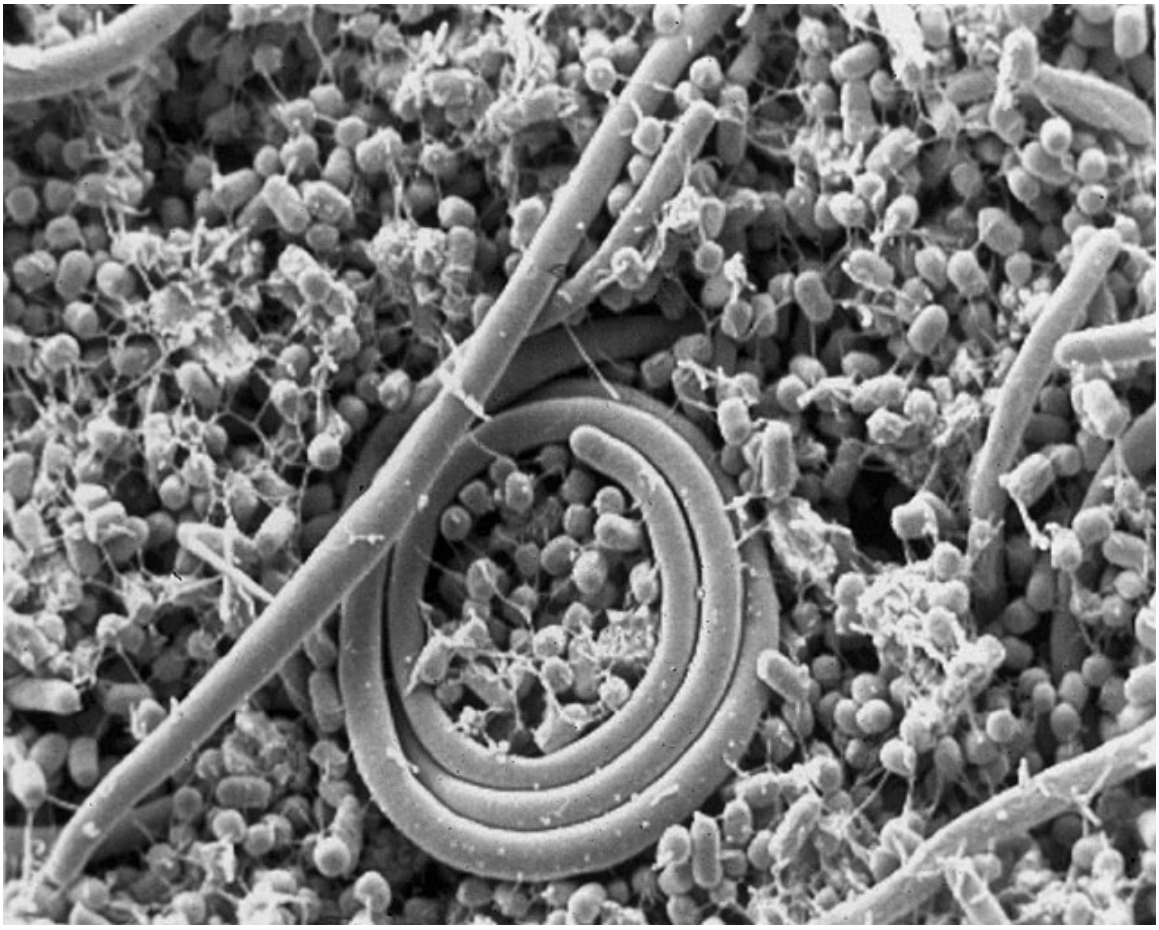


Figure 1. Example of signaling between bacteria. Salmonella enteritidis uses acyl-homoserine lactone for Quorum sensing

Cell signaling has been most extensively studied in the context of human diseases and signaling between cells of a single organism. However, cell signaling may also occur between the cells of two different organisms. In many mammals, early embryo cells exchange signals with cells of the uterus. In the human gastrointestinal tract, bacteria exchange signals with each other and with human epithelial and immune system cells. For the yeast *Saccharomyces cerevisiae* during mating, some cells send a peptide signal (mating factor *pheromones*) into their environment. The mating factor peptide may bind to a cell surface receptor on other yeast cells and induce them to prepare for mating.

Types of signals

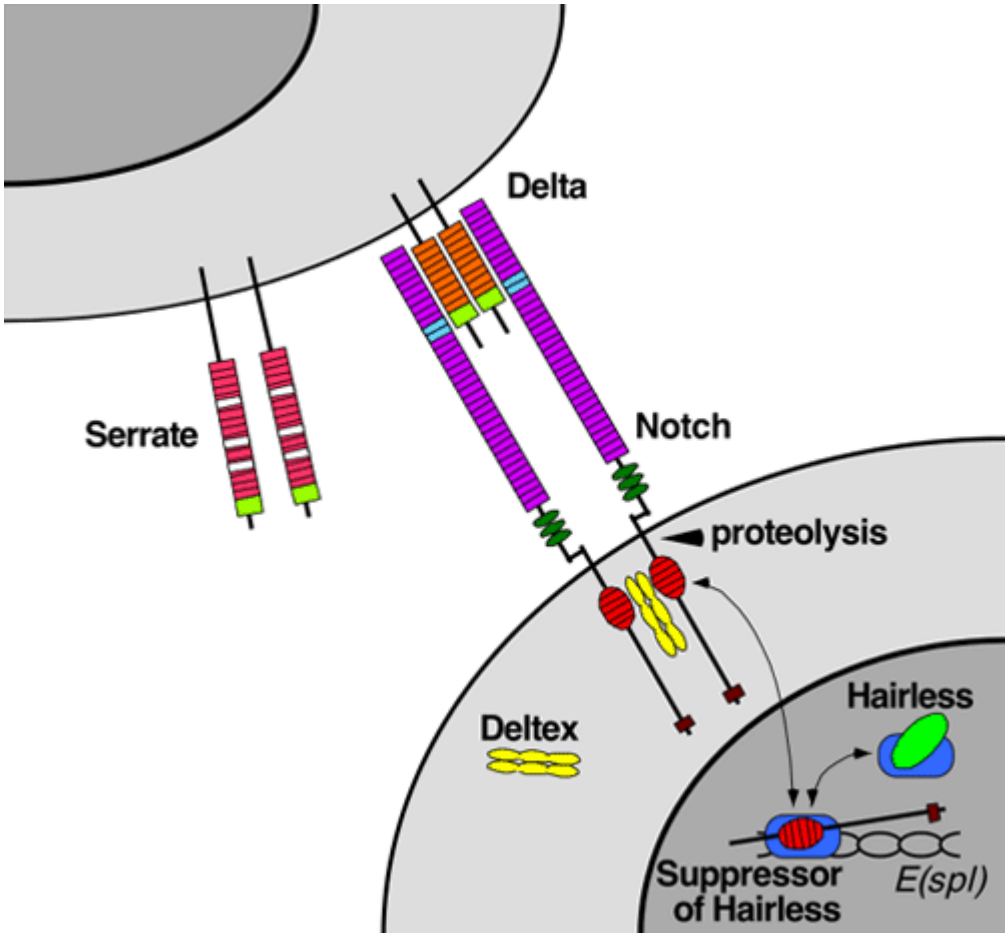


Figure 2. Notch-mediated juxtacrine signal between adjacent cells.

Cells communicate with each other via direct contact (juxtacrine signaling), over short distances (paracrine signaling), or over large distances and/or scales (endocrine signaling).

Some cell-to-cell communication requires direct cell-cell contact. Some cells can form gap junctions that connect their cytoplasm to the cytoplasm of adjacent cells. In cardiac muscle, gap junctions between adjacent cells allows for action potential propagation from the cardiac pacemaker region of the heart to spread and coordinately cause contraction of the heart.

The Notch signaling mechanism is an example of juxtacrine signalling (also known as contact-dependent signaling) in which two adjacent cells must make physical contact in order to communicate. This requirement for direct contact allows for very precise control of cell differentiation during embryonic development. In the worm *Caenorhabditis elegans*, two cells of the developing gonad each have an equal chance of terminally differentiating or becoming a uterine precursor cell that continues to divide. The choice

of which cell continues to divide is controlled by competition of cell surface signals. One cell will happen to produce more of a cell surface protein that activates the Notch receptor on the adjacent cell. This activates a feedback loop or system that reduces Notch expression in the cell that will differentiate and that increases Notch on the surface of the cell that continues as a stem cell.

Many cell signals are carried by molecules that are released by one cell and move to make contact with another cell. *Endocrine* signals are called hormones. Hormones are produced by endocrine cells and they travel through the blood to reach all parts of the body. Specificity of signaling can be controlled if only some cells can respond to a particular hormone. *Paracrine* signals such as retinoic acid target only cells in the vicinity of the emitting cell. Neurotransmitters represent another example of a paracrine signal. Some signaling molecules can function as both a hormone and a neurotransmitter. For example, epinephrine and norepinephrine can function as hormones when released from the adrenal gland and are transported to the heart by way of the blood stream. Norepinephrine can also be produced by neurons to function as a neurotransmitter within the brain. Estrogen can be released by the ovary and function as a hormone or act locally via paracrine or autocrine signaling. Active species of oxygen and nitric oxide can also act as cellular messengers. This process is dubbed redox signaling.

Receptors for cell moves

Cells receive information from their environment through a class of proteins known as receptors. Notch is a cell surface protein that functions as a receptor. Animals have a small set of genes that code for signaling proteins that interact specifically with Notch receptors and stimulate a response in cells that express Notch on their surface. Molecules that activate (or, in some cases, inhibit) receptors can be classified as hormones, neurotransmitters, cytokines, growth factors but all of these are called receptor ligands. The details of ligand-receptor interactions are fundamental to cell signaling.

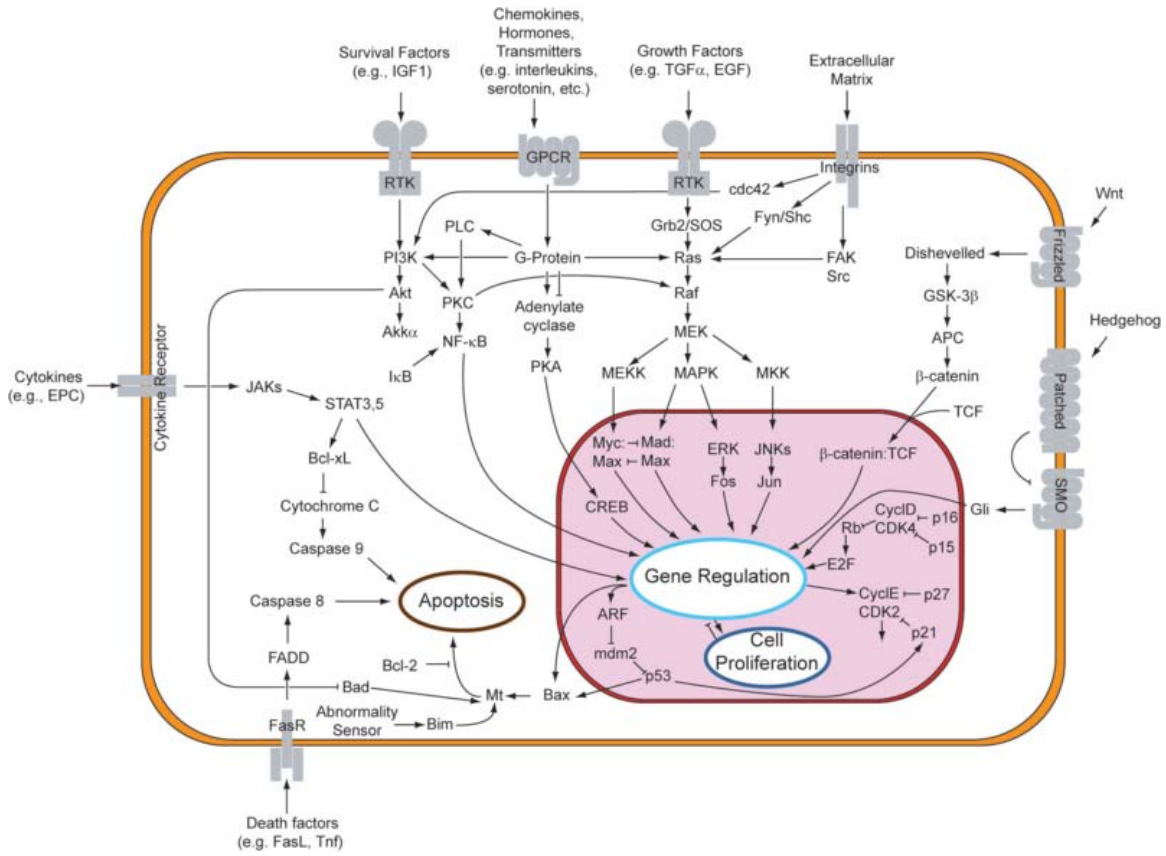
As shown in Figure 2 (above, left), Notch acts as a receptor for ligands that are expressed on adjacent cells. While many receptors are cell surface proteins, some are found inside cells. For example, oestrogen is a hydrophobic molecule that can pass through the lipid bilayer of cell surface membranes. Oestrogen receptors inside cells of the uterus can be activated by oestrogen that comes from the ovaries, enters the target cells, and binds to oestrogen receptors.

A number of transmembrane receptors for molecules that include peptide hormones and of intracellular receptors for steroid hormones exist, giving to a cell the ability to respond to a great number of hormonal and pharmacological stimuli. In diseases, often, proteins that interact with receptors are aberrantly activated, resulting in constitutively activated downstream signals.

For several types of intercellular signaling molecules that are unable to permeate the hydrophobic cell membrane due to their hydrophilic nature, the target receptor is

expressed on the membrane. When such signaling molecule activates its receptor, the signal is carried into the cell usually by means of a second messenger such as cAMP.

Signaling pathways



Overview of signal transduction pathways.

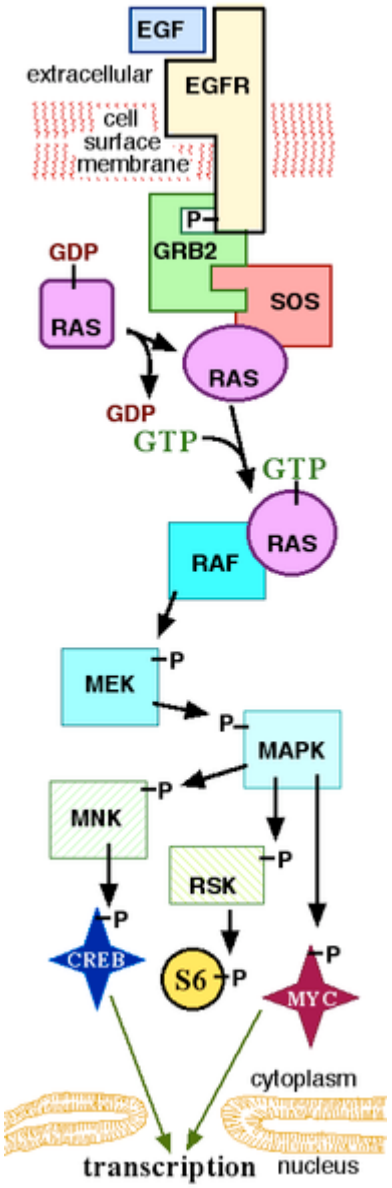


Figure 3. Diagram showing key components of a signal transduction pathway.

In some cases, receptor activation caused by ligand binding to a receptor is directly coupled to the cell's response to the ligand. For example, the neurotransmitter GABA can activate a cell surface receptor that is part of an ion channel. GABA binding to a GABA A receptor on a neuron opens a chloride-selective ion channel that is part of the receptor. GABA A receptor activation allows negatively-charged chloride ions to move into the neuron, which inhibits the ability of the neuron to produce action potentials. However, for many cell surface receptors, ligand-receptor interactions are not directly linked to the cell's response. The activated receptor must first interact with other proteins inside the cell before the ultimate physiological effect of the ligand on the cell's behavior is produced. Often, the behavior of a chain of several interacting cell proteins is altered

following receptor activation. The entire set of cell changes induced by receptor activation is called a signal transduction mechanism or pathway.

In the case of Notch-mediated signaling, the signal transduction mechanism can be relatively simple. As shown in Figure 2 (above, left), activation of Notch can cause the Notch protein to be altered by a protease. Part of the Notch protein is released from the cell surface membrane and can act to change the pattern of gene transcription in the cell nucleus. This causes the responding cell to make different proteins, resulting in an altered pattern of cell behavior. Cell signaling research involves studying the spatial and temporal dynamics of both receptors and the components of signaling pathways that are activated by receptors in various cell types.

A more complex signal transduction pathway is shown in Figure 3. This pathway involves changes of protein-protein interactions inside the cell, induced by an external signal. Many growth factors bind to receptors at the cell surface and stimulate cells to progress through the cell cycle and divide. Several of these receptors are kinases that start to phosphorylate themselves and other proteins when binding to a ligand. This phosphorylation can generate a binding site for a different protein and thus induce protein-protein interaction. In Figure 3, the ligand (called epidermal growth factor (EGF)) binds to the receptor (called EGFR). This activates the receptor to phosphorylate itself. The phosphorylated receptor binds to an adaptor protein (GRB2), which couples the signal to further downstream signaling processes. For example, one of the signal transduction pathways that are activated is called the mitogen-activated protein kinase (MAPK) pathway. The signal transduction component labeled as "MAPK" in the pathway was originally called "ERK," so the pathway is called the MAPK/ERK pathway. The MAPK protein is an enzyme, a protein kinase that can attach phosphate to target proteins such as the transcription factor MYC and, thus, alter gene transcription and, ultimately, cell cycle progression. Many cellular proteins are activated downstream of the growth factor receptors (such as EGFR) that initiate this signal transduction pathway.

Some signaling transduction pathways respond differently depending on the amount of signaling received by the cell. For instance, the hedgehog protein activates different genes, depending on the amount of hedgehog protein present.

Complex multi-component signal transduction pathways provide opportunities for feedback, signal amplification, and interactions inside one cell between multiple signals and signaling pathways.

Classification of intercellular communication

Within endocrinology (the study of intercellular signalling in animals) and the endocrine system, intercellular signalling is subdivided into the following classifications:

- *Intracrine* signals are produced within the target cell.

- *Autocrine* signals target the cell itself. Sometimes autocrine cells can target cells close by if they are the same type of cell as the emitting cell. An example of this are immune cells.
- *Juxtacrine* signals target adjacent (touching) cells. These signals are transmitted along cell membranes via protein or lipid components integral to the membrane and are capable of affecting either the emitting cell or cells immediately adjacent.
- *Paracrine* signals target cells in the vicinity of the emitting cell. Neurotransmitters represent an example.
- *Endocrine* signals target distant cells. Endocrine cells produce hormones that travel through the blood to reach all parts of the body.

Chapter- 8

Cell Migration

Cell migration is a central process in the development and maintenance of multicellular organisms. Tissue formation during embryonic development, wound healing and immune responses all require the orchestrated movement of cells in particular directions to specific locations. Errors during this process have serious consequences, including mental retardation, vascular disease, tumor formation and metastasis. An understanding of the mechanism by which cells migrate may lead to the development of novel therapeutic strategies for controlling, for example, invasive tumour cells. Cells often migrate in response to, and towards, specific external signals, a process called chemotaxis.

Studying cell migration

The migration of single mammalian cells is usually viewed in the microscope as the cells move randomly on a glass slide. As the actual movement is very slow — usually a few micrometers/minute — time-lapse films are taken so that a speeded up movie can be viewed. This shows that, although the shape of a moving cell varies considerably, its leading front has a characteristic behaviour. This region of the cell is highly active, sometimes spreading forwards quickly, sometimes retracting, sometimes ruffling or bubbling. It is generally accepted that the leading front is the main motor which pulls the cell forward.

Common features

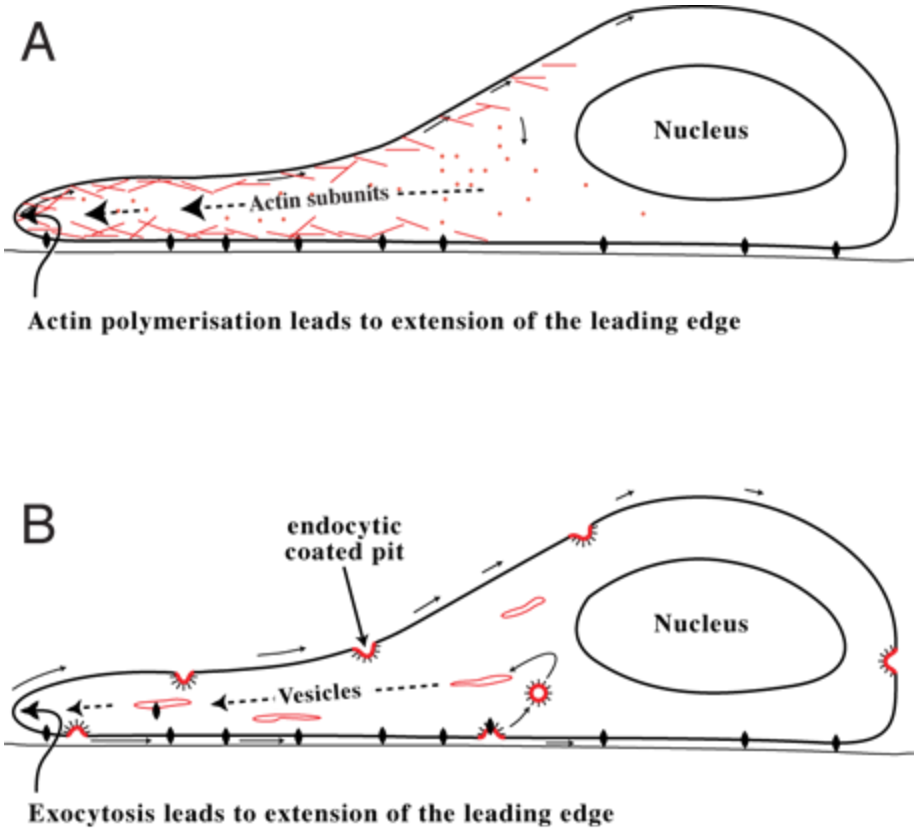
There is still great uncertainty of how cell migration really works. However, because the locomotion of all mammalian cells (except sperm) has several common features, the underlying processes are believed to be similar. The two main constant features are:

1. the behaviour of the leading front and
2. the observation that any debris on the dorsal surface of the cell moves backwards on the cell's surface towards its trailing end. The latter feature is most easily observed when aggregates of a surface molecule are cross-linked with a fluorescent antibody or when small beads become artificially bound to the front of the cell.

Besides mammalian cells, many other eukaryotic cells appear to move in a similar way. One of the most valuable model creatures for studying locomotion and chemotaxis is the amoeba Dictyostelium discoideum because they move more quickly than most mammalian cells grown in the lab and they chemotax towards cyclic AMP. In addition, they have a haploid genome which assists understanding the role of a particular gene product in movement.

Molecular processes at the front

There are two main theories for how the cell advances its front edge: the cytoskeletal model and membrane flow model. It is possible that both underlying processes contribute to cell extension.



Two different models for how cells move. A) Cytoskeletal model. B) Membrane Flow Model

Cytoskeletal model (A)

Experimentally it is found that the cell's front is a site of rapid actin polymerisation: soluble actin monomers polymerise there to form filaments. This has led to the view that it is the formation of these actin filaments which pushes the leading front forward and is the main motile force for advancing the cell's front. In addition, cytoskeletal elements are able to interact extensively and intimately with a cell's plasma membrane.

Membrane flow model (B)

Studies have also shown that the front is the site at which membrane is returned to the cell surface from internal membrane pools at the end of the endocytic cycle. This has led to the view that extension of the leading edge occurs primarily by addition of membrane at the front of the cell. If so, the actin filaments which form at the front might stabilize the added membrane so that a structured extension, or lamella, is formed rather than the cell blowing bubbles (or "blebs") at its front. For a cell to move, it is necessary to bring a fresh supply of feet — those molecules, called integrins, which attach a cell to the surface on which it is crawling — to the front. It is likely that these feet are endocytosed towards the rear of the cell and brought to the cell's front by exocytosis, to be reused to form new attachments to the substrate.

The nucleus and rear

Given that a cell's front advances, what about the rest of the cell? Is it simply dragged forward, like a sack? We do not know, but there are suggestions that the nucleus and perhaps other large structures inside the cell may also be pulled forward by actin filaments. In addition, it may be that the rear of the cell actively contracts, as it is here that, in some cells, the major contractile protein myosin is found.

Mutants

Insight into how complex biological processes work can often be gleaned from a study of mutations. In the case of the intracellular mechanisms underlying cell movement, this has been largely unsuccessful. Thus, although many mutants are known in Drosophila which affect migratory processes, these tend to fall into two groups: transcription factors (such as *slow border cells* (*slbo*) which affects the migration of the border cells) or key regulator proteins (such as C-Jun N-terminal kinases (JNK) which controls dorsal closure). These, however, tell us little about how cells actually move.

Another major source of mutants is the haploid amoeba Dictyostelium. Many single copy genes associated with cytoskeletal function have been deleted: these mutants usually have only a weak phenotype, suggesting either that these genes are not required for locomotion or that there are multiple mechanisms by which cells can move. However, temperature-sensitive mutants in the genes for N-ethylmaleimide sensitive fusion protein (NSF) and Sec1 rapidly block cell migration indicating that the NSF protein and Sec1p are both required for some aspects of cell movement. NSF is known to function in intracellular membrane fusion; Sec1p in yeast is required for polarised exocytosis.

Polarity in migrating cells

Migrating cells clearly have a polarity: a front and a back. Without it, they would move in all directions at once, or spread. How this arrow is formulated at a molecular level inside a cell is unknown. In a cell which is meandering in a random way, the front can easily give way to become passive as some other region, or regions, of the cell form(s) a new

front. In chemotaxing cells, the stability of the front appears enhanced as the cell advances towards a higher concentration of the stimulating chemical. This polarity is reflected at a molecular level by a restriction of certain molecules to particular regions of the inner cell surface: thus the phospholipid PIP3 and activated Rac and CDC42 are found at the front of the cell, whereas Rho GTPase and PTEN are found towards the rear.

It is believed that microtubules and filamentous actin are important for establishing and maintaining a cell's polarity. Thus, drugs which destroy microtubules disrupt the polarity of many cells: if the cell is attached to a substratum, they often become round and flat. Drugs which destroy actin filaments have multiple and complex effects, reflecting the wide role that these filaments play in many cell processes. It may be that, as part of the locomotory process, membrane vesicles are transported along these filaments to the cell's front. In chemotaxing cells, the increased persistence of migration towards the target may result from an increased stability of the arrangement of the filamentous structures inside the cell and which determine its polarity. In turn, these filamentous structures may be arranged inside the cell according to how molecules like PIP3 and PTEN are arranged on the inner cell surface. And where these are located appears in turn to be determined by the chemoattractant signals as these impinge on specific receptors on the cell's outer surface.

Chapter- 9

Transcription (Genetics)

Transcription is the process of creating a complementary RNA copy of a sequence of DNA. Both RNA and DNA are nucleic acids, which use base pairs of nucleotides as a complementary language that can be converted back and forth from DNA to RNA by the action of the correct enzymes. During transcription, a DNA sequence is read by RNA polymerase, which produces a complementary, antiparallel RNA strand. As opposed to DNA replication, transcription results in an RNA complement that includes uracil (U) in all instances where thymine (T) would have occurred in a DNA complement.

Transcription can be explained easily in 4 or 5 simple steps, each moving like a wave along the DNA.

1. DNA unwinds/"unzips" as the Hydrogen Bonds Break.
2. The free nucleotides of the RNA, pair with complementary DNA bases.
3. RNA sugar-phosphate backbone forms. (Aided by RNA Polymerase.)
4. Hydrogen bonds of the untwisted RNA+DNA "ladder" break, freeing the new RNA.
5. If the cell has a nucleus, the RNA is further processed and then moves through the small nuclear pores to the cytoplasm.

Transcription is the first step leading to gene expression. The stretch of DNA transcribed into an RNA molecule is called a *transcription unit* and encodes at least one gene. If the gene transcribed encodes a protein, the result of transcription is messenger RNA (mRNA), which will then be used to create that protein via the process of translation. Alternatively, the transcribed gene may encode for either ribosomal RNA (rRNA) or transfer RNA (tRNA), other components of the protein-assembly process, or other ribozymes.

A DNA transcription unit encoding for a protein contains not only the sequence that will eventually be directly translated into the protein (the *coding sequence*) but also *regulatory sequences* that direct and regulate the synthesis of that protein. The regulatory sequence before (upstream from) the coding sequence is called the five prime untranslated region (5'UTR), and the sequence following (downstream from) the coding sequence is called the three prime untranslated region (3'UTR).

Transcription has some proofreading mechanisms, but they are fewer and less effective than the controls for copying DNA; therefore, transcription has a lower copying fidelity than DNA replication.

As in DNA replication, DNA is read from 3' → 5' during transcription. Meanwhile, the complementary RNA is created from the 5' → 3' direction. This means its 5' end is created first in base pairing. Although DNA is arranged as two antiparallel strands in a double helix, only one of the two DNA strands, called the template strand, is used for transcription. This is because RNA is only single-stranded, as opposed to double-stranded DNA. The other DNA strand is called the coding strand, because its sequence is the same as the newly created RNA transcript (except for the substitution of uracil for thymine). The use of only the 3' → 5' strand eliminates the need for the Okazaki fragments seen in DNA replication.

Transcription is divided into 5 stages: *pre-initiation*, *initiation*, *promoter clearance*, *elongation* and *termination*.

Major steps

Pre-initiation

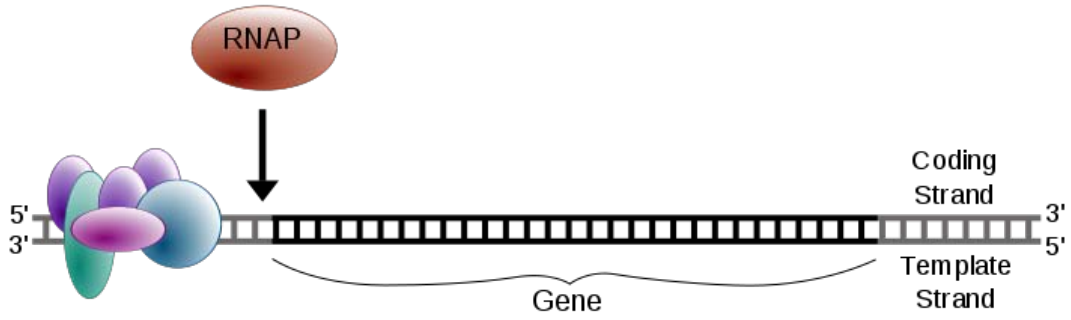
In eukaryotes, RNA polymerase, and therefore the initiation of transcription, requires the presence of a core promoter sequence in the DNA. Promoters are regions of DNA that promote transcription and, in eukaryotes, are found at -30, -75, and -90 base pairs upstream from the start site of transcription. Core promoters are sequences within the promoter that are essential for transcription initiation. RNA polymerase is able to bind to core promoters in the presence of various specific transcription factors.

The most common type of core promoter in eukaryotes is a short DNA sequence known as a TATA box, found 25-30 base pairs upstream from the start site of transcription. The TATA box, as a core promoter, is the binding site for a transcription factor known as TATA-binding protein (TBP), which is itself a subunit of another transcription factor, called Transcription Factor II D (TFIID). After TFIID binds to the TATA box via the TBP, five more transcription factors and RNA polymerase combine around the TATA box in a series of stages to form a preinitiation complex. One transcription factor, DNA helicase, has helicase activity and so is involved in the separating of opposing strands of double-stranded DNA to provide access to a single-stranded DNA template. However, only a low, or basal, rate of transcription is driven by the preinitiation complex alone. Other proteins known as activators and repressors, along with any associated coactivators or corepressors, are responsible for modulating transcription rate.

Thus, preinitiation complex contains: 1. Core Promoter Sequence 2. Transcription Factors 3. DNA Helicase 4. RNA Polymerase 5. Activators and Repressors The transcription preinitiation in archaea is, in essence, homologous to that of eukaryotes, but is much less complex. The archaeal preinitiation complex assembles at a TATA-box

binding site; however, in archaea, this complex is composed of only RNA polymerase II, TBP, and TFB (the archaeal homologue of eukaryotic transcription factor II B (TFIIB)).

Initiation



Simple diagram of transcription initiation. RNAP = RNA polymerase

In bacteria, transcription begins with the binding of RNA polymerase to the promoter in DNA. RNA polymerase is a core enzyme consisting of five subunits: 2 α subunits, 1 β subunit, 1 β' subunit, and 1 ω subunit. At the start of initiation, the core enzyme is associated with a sigma factor that aids in finding the appropriate -35 and -10 base pairs downstream of promoter sequences.

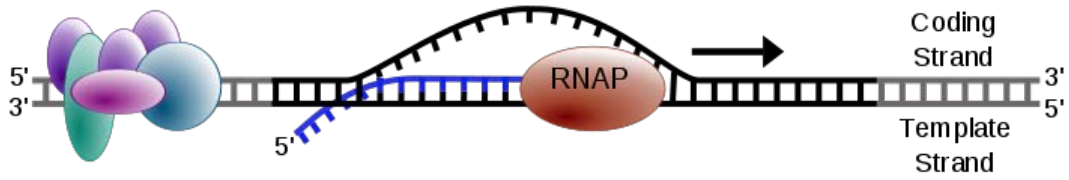
Transcription initiation is more complex in eukaryotes. Eukaryotic RNA polymerase does not directly recognize the core promoter sequences. Instead, a collection of proteins called transcription factors mediate the binding of RNA polymerase and the initiation of transcription. Only after certain transcription factors are attached to the promoter does the RNA polymerase bind to it. The completed assembly of transcription factors and RNA polymerase bind to the promoter, forming a transcription initiation complex. Transcription in the archaea domain is similar to transcription in eukaryotes.

Promoter clearance

After the first bond is synthesized, the DNA polymerase must clear the promoter. During this time there is a tendency to release the RNA transcript and produce truncated transcripts. This is called *abortive initiation* and is common for both eukaryotes and prokaryotes. Abortive initiation continues to occur until the σ factor rearranges, resulting in the transcription elongation complex (which gives a 35 bp moving footprint). The σ factor is released before 80 nucleotides of mRNA are synthesized. Once the transcript reaches approximately 23 nucleotides, it no longer slips and elongation can occur. This, like most of the remainder of transcription, is an energy-dependent process, consuming adenosine triphosphate (ATP).

Promoter clearance coincides with phosphorylation of serine 5 on the carboxy terminal domain of RNA Pol in eukaryotes, which is phosphorylated by TFIIH.

Elongation



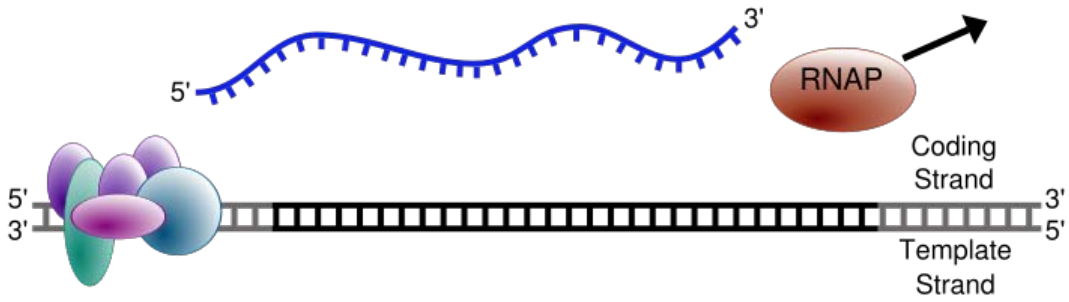
Simple diagram of transcription elongation

One strand of the DNA, the *template strand* (or noncoding strand), is used as a template for RNA synthesis. As transcription proceeds, RNA polymerase traverses the template strand and uses base pairing complementarity with the DNA template to create an RNA copy. Although RNA polymerase traverses the template strand from 3' → 5', the coding (non-template) strand and newly-formed RNA can also be used as reference points, so transcription can be described as occurring 5' → 3'. This produces an RNA molecule from 5' → 3', an exact copy of the coding strand (except that thymines are replaced with uracils, and the nucleotides are composed of a ribose (5-carbon) sugar where DNA has deoxyribose (one less oxygen atom) in its sugar-phosphate backbone).

Unlike DNA replication, mRNA transcription can involve multiple RNA polymerases on a single DNA template and multiple rounds of transcription (amplification of particular mRNA), so many mRNA molecules can be rapidly produced from a single copy of a gene.

Elongation also involves a proofreading mechanism that can replace incorrectly incorporated bases. In eukaryotes, this may correspond with short pauses during transcription that allow appropriate RNA editing factors to bind. These pauses may be intrinsic to the RNA polymerase or due to chromatin structure.

Termination



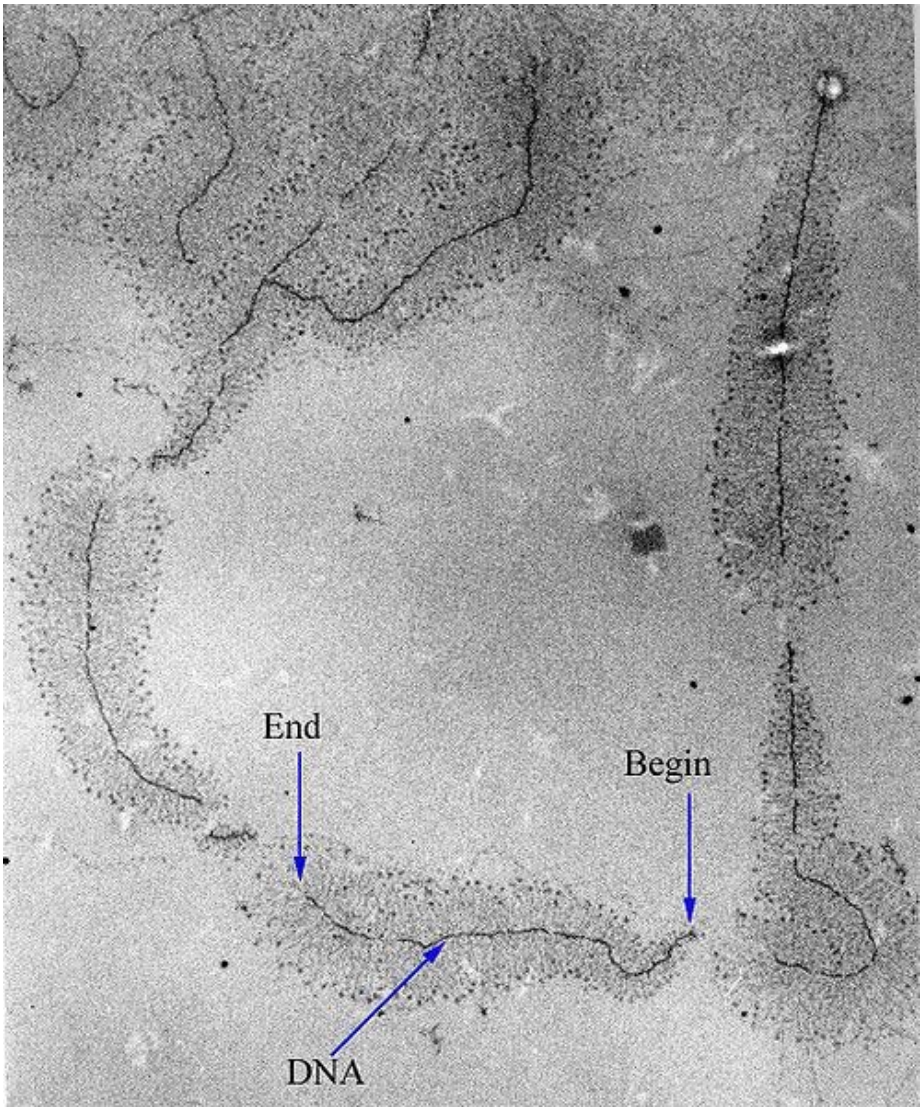
Simple diagram of transcription termination

Bacteria use two different strategies for transcription termination. In Rho-independent transcription termination, RNA transcription stops when the newly synthesized RNA

molecule forms a G-C-rich hairpin loop followed by a run of Us. When the hairpin forms, the mechanical stress breaks the weak rU-dA bonds, now filling the DNA-RNA hybrid. This pulls the poly-U transcript out of the active site of the RNA polymerase, in effect, terminating transcription. In the "Rho-dependent" type of termination, a protein factor called "Rho" destabilizes the interaction between the template and the mRNA, thus releasing the newly synthesized mRNA from the elongation complex.

Transcription termination in eukaryotes is less understood but involves cleavage of the new transcript followed by template-independent addition of *A*s at its new 3' end, in a process called polyadenylation.

Measuring and detecting transcription



Electron micrograph of the ribosomal transcription process. The forming mRNA strands are visible as branches from the main DNA strand.

Transcription can be measured and detected in a variety of ways:

- Nuclear Run-on assay: measures the relative abundance of newly formed transcripts
- RNase protection assay and ChIP-Chip of RNAP: detect active transcription sites
- RT-PCR: measures the absolute abundance of total or nuclear RNA levels, which may however differ from transcription rates
- DNA microarrays: measures the relative abundance of the global total or nuclear RNA levels; however, these may differ from transcription rates
- In situ hybridization: detects the presence of a transcript
- MS2 tagging: by incorporating RNA stem loops, such as MS2, into a gene, these become incorporated into newly synthesized RNA. The stem loops can then be detected using a fusion of GFP and the MS2 coat protein, which has a high affinity, sequence-specific interaction with the MS2 stem loops. The recruitment of GFP to the site of transcription is visualised as a single fluorescent spot. This remarkable new approach has revealed that transcription occurs in discontinuous bursts, or pulses. With the notable exception of in situ techniques, most other methods provide cell population averages, and are not capable of detecting this fundamental property of genes.
- Northern blot: the traditional method, and until the advent of RNA-Seq, the most quantitative
- RNA-Seq: applies next-generation sequencing techniques to sequence whole transcriptomes, which allows the measurement of relative abundance of RNA, as well as the detection of additional variations such as fusion genes, post-translational edits and novel splice sites

Transcription factories

Active transcription units are clustered in the nucleus, in discrete sites called transcription factories or euchromatin. Such sites can be visualized by allowing engaged polymerases to extend their transcripts in tagged precursors (Br-UTP or Br-U) and immuno-labeling the tagged nascent RNA. Transcription factories can also be localized using fluorescence in situ hybridization or marked by antibodies directed against polymerases. There are ~10,000 factories in the nucleoplasm of a HeLa cell, among which are ~8,000 polymerase II factories and ~2,000 polymerase III factories. Each polymerase II factory contains ~8 polymerases. As most active transcription units are associated with only one polymerase, each factory usually contains ~8 different transcription units. These units might be associated through promoters and/or enhancers, with loops forming a ‘cloud’ around the factor.

History

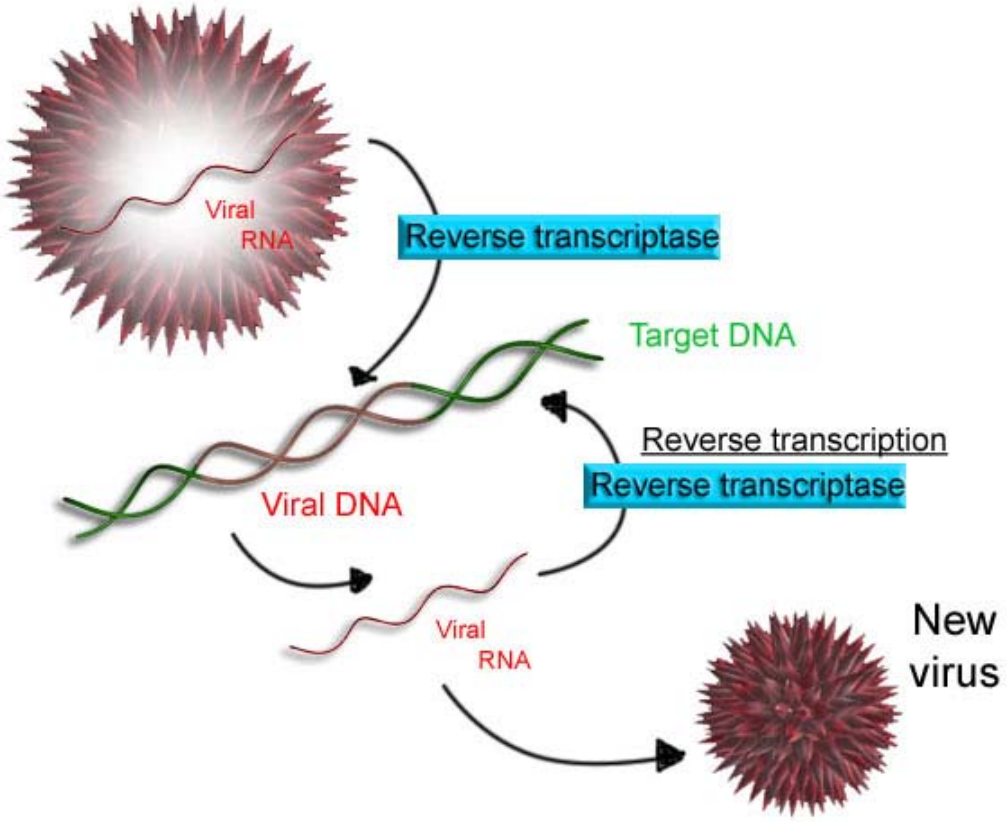
A molecule that allows the genetic material to be realized as a protein was first hypothesized by François Jacob and Jacques Monod. RNA synthesis by RNA polymerase

was established *in vitro* by several laboratories by 1965; however, the RNA synthesized by these enzymes had properties that suggested the existence of an additional factor needed to terminate transcription correctly.

In 1972, Walter Fiers became the first person to actually prove the existence of the terminating enzyme.

Roger D. Kornberg won the 2006 Nobel Prize in Chemistry "for his studies of the molecular basis of eukaryotic transcription".

Reverse transcription



Scheme of reverse transcription

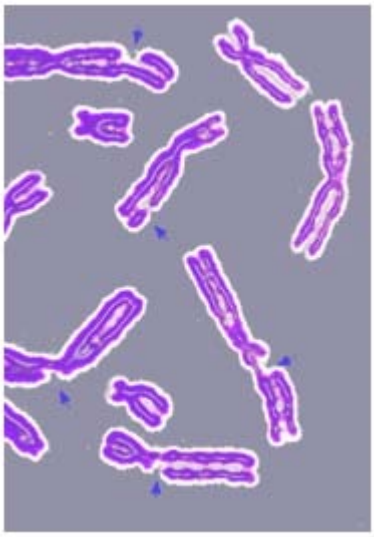
Some viruses (such as HIV, the cause of AIDS), have the ability to transcribe RNA into DNA. HIV has an RNA genome that is duplicated into DNA. The resulting DNA can be merged with the DNA genome of the host cell. The main enzyme responsible for synthesis of DNA from an RNA template is called reverse transcriptase. In the case of HIV, reverse transcriptase is responsible for synthesizing a complementary DNA strand (cDNA) to the viral RNA genome. An associated enzyme, ribonuclease H, digests the RNA strand, and reverse transcriptase synthesises a complementary strand of DNA to form a double helix DNA structure. This cDNA is integrated into the host cell's genome via another enzyme (integrase) causing the host cell to generate viral proteins that

reassemble into new viral particles. Subsequent to this, the host cell undergoes programmed cell death, apoptosis.

Some eukaryotic cells contain an enzyme with reverse transcription activity called telomerase. Telomerase is a reverse transcriptase that lengthens the ends of linear chromosomes. Telomerase carries an RNA template from which it synthesizes DNA repeating sequence, or "junk" DNA. This repeated sequence of DNA is important because, every time a linear chromosome is duplicated, it is shortened in length. With "junk" DNA at the ends of chromosomes, the shortening eliminates some of the non-essential, repeated sequence rather than the protein-encoding DNA sequence farther away from the chromosome end. Telomerase is often activated in cancer cells to enable cancer cells to duplicate their genomes indefinitely without losing important protein-coding DNA sequence. Activation of telomerase could be part of the process that allows cancer cells to become *immortal*. However, the true *in vivo* significance of telomerase has still not been empirically proven.

Chapter- 10

DNA Repair



DNA damage resulting in multiple broken chromosomes

DNA repair refers to a collection of processes by which a cell identifies and corrects damage to the DNA molecules that encode its genome. In human cells, both normal metabolic activities and environmental factors such as UV light and radiation can cause DNA damage, resulting in as many as 1 million individual molecular lesions per cell per day. Many of these lesions cause structural damage to the DNA molecule and can alter or eliminate the cell's ability to transcribe the gene that the affected DNA encodes. Other lesions induce potentially harmful mutations in the cell's genome, which affect the survival of its daughter cells after it undergoes mitosis. As a consequence, the DNA repair process is constantly active as it responds to damage in the DNA structure. When normal repair processes fail, and when cellular apoptosis does not occur, irreparable DNA damage may occur, including double-strand breaks and DNA crosslinkages.

The rate of DNA repair is dependent on many factors, including the cell type, the age of the cell, and the extracellular environment. A cell that has accumulated a large amount of

DNA damage, or one that no longer effectively repairs damage incurred to its DNA, can enter one of three possible states:

1. an irreversible state of dormancy, known as senescence
2. cell suicide, also known as apoptosis or programmed cell death
3. unregulated cell division, which can lead to the formation of a tumor that is cancerous

The DNA repair ability of a cell is vital to the integrity of its genome and thus to its normal functioning and that of the organism. Many genes that were initially shown to influence life span have turned out to be involved in DNA damage repair and protection. Failure to correct molecular lesions in cells that form gametes can introduce mutations into the genomes of the offspring and thus influence the rate of evolution.

DNA damage

DNA damage, due to environmental factors and normal metabolic processes inside the cell, occurs at a rate of 1,000 to 1,000,000 molecular lesions per cell per day. While this constitutes only 0.000165% of the human genome's approximately 6 billion bases (3 billion base pairs), unrepaired lesions in critical genes (such as tumor suppressor genes) can impede a cell's ability to carry out its function and appreciably increase the likelihood of tumor formation.

The vast majority of DNA damage affects the primary structure of the double helix; that is, the bases themselves are chemically modified. These modifications can in turn disrupt the molecules' regular helical structure by introducing non-native chemical bonds or bulky adducts that do not fit in the standard double helix. Unlike proteins and RNA, DNA usually lacks tertiary structure and therefore damage or disturbance does not occur at that level. DNA is, however, supercoiled and wound around "packaging" proteins called histones (in eukaryotes), and both superstructures are vulnerable to the effects of DNA damage.

Sources of damage

DNA damage can be subdivided into two main types:

1. endogenous damage such as attack by reactive oxygen species produced from normal metabolic byproducts (spontaneous mutation), especially the process of oxidative deamination
    1. also includes replication errors
2. exogenous damage caused by external agents such as
    1. ultraviolet [UV 200-300nm] radiation from the sun
    2. other radiation frequencies, including x-rays and gamma rays
    3. hydrolysis or thermal disruption
    4. certain plant toxins

5. human-made mutagenic chemicals, especially aromatic compounds that act as DNA intercalating agents
6. cancer chemotherapy and radiotherapy
7. viruses

The replication of damaged DNA before cell division can lead to the incorporation of wrong bases opposite damaged ones. Daughter cells that inherit these wrong bases carry mutations from which the original DNA sequence is unrecoverable (except in the rare case of a back mutation, for example, through gene conversion).

Types of damage

There are five main types of damage to DNA due to endogenous cellular processes:

1. *oxidation* of bases [e.g. 8-oxo-7,8-dihydroguanine (8-oxoG)] and generation of DNA strand interruptions from reactive oxygen species,
2. *alkylation* of bases (usually methylation), such as formation of 7-methylguanine, 1-methyladenine, 6-O-Methylguanine
3. *hydrolysis* of bases, such as deamination, depurination, and depyrimidination.
4. "bulky adduct formation" (i.e., benzo[a]pyrene diol epoxide-dG adduct)
5. *mismatch* of bases, due to errors in DNA replication, in which the wrong DNA base is stitched into place in a newly forming DNA strand, or a DNA base is skipped over or mistakenly inserted.

Damage caused by exogenous agents comes in many forms. Some examples are:

1. *UV-B light* causes crosslinking between adjacent cytosine and thymine bases creating *pyrimidine dimers*. This is called direct DNA damage.
2. *UV-A light* creates mostly free radicals. The damage caused by free radicals is called indirect DNA damage.
3. *Ionizing radiation* such as that created by radioactive decay or in *cosmic rays* causes breaks in DNA strands. Low-level ionizing radiation may induce irreparable DNA damage (leading to replicational and transcriptional errors needed for neoplasia or may trigger viral interactions) leading to pre-mature aging and cancer.
4. *Thermal disruption* at elevated temperature increases the rate of depurination (loss of purine bases from the DNA backbone) and single-strand breaks. For example, hydrolytic depurination is seen in the thermophilic bacteria, which grow in hot springs at 40-80 °C. The rate of depurination (300 purine residues per genome per generation) is too high in these species to be repaired by normal repair machinery, hence a possibility of an adaptive response cannot be ruled out.
5. *Industrial chemicals* such as vinyl chloride and hydrogen peroxide, and environmental chemicals such as polycyclic hydrocarbons found in smoke, soot and tar create a huge diversity of DNA adducts- ethenobases, oxidized bases, alkylated phosphotriesters and Crosslinking of DNA just to name a few.

UV damage, alkylation/methylation, X-ray damage and oxidative damage are examples of induced damage. Spontaneous damage can include the loss of a base, deamination, sugar ring puckering and tautomeric shift.

Nuclear versus mitochondrial DNA damage

In human cells, and eukaryotic cells in general, DNA is found in two cellular locations - inside the nucleus and inside the mitochondria. Nuclear DNA (nDNA) exists as chromatin during non-replicative stages of the cell cycle and is condensed into aggregate structures known as chromosomes during cell division. In either state the DNA is highly compacted and wound up around bead-like proteins called histones. Whenever a cell needs to express the genetic information encoded in its nDNA the required chromosomal region is unravelled, genes located therein are expressed, and then the region is condensed back to its resting conformation. Mitochondrial DNA (mtDNA) is located inside mitochondria organelles, exists in multiple copies, and is also tightly associated with a number of proteins to form a complex known as the nucleoid. Inside mitochondria, reactive oxygen species (ROS), or free radicals, byproducts of the constant production of adenosine triphosphate (ATP) via oxidative phosphorylation, create a highly oxidative environment that is known to damage mtDNA. A critical enzyme in counteracting the toxicity of these species is superoxide dismutase, which is present in both the mitochondria and cytoplasm of eukaryotic cells.

Senescence and apoptosis

Senescence, an irreversible state in which the cell no longer divides, is a protective response to the shortening of the chromosome ends. The telomeres are long regions of repetitive noncoding DNA that cap chromosomes and undergo partial degradation each time a cell undergoes division. In contrast, quiescence is a reversible state of cellular dormancy that is unrelated to genome damage. Senescence in cells may serve as a functional alternative to apoptosis in cases where the physical presence of a cell for spatial reasons is required by the organism, which serves as a "last resort" mechanism to prevent a cell with damaged DNA from replicating inappropriately in the absence of pro-growth cellular signaling. Unregulated cell division can lead to the formation of a tumor, which is potentially lethal to an organism. Therefore, the induction of senescence and apoptosis is considered to be part of a strategy of protection against cancer.

DNA damage and mutation

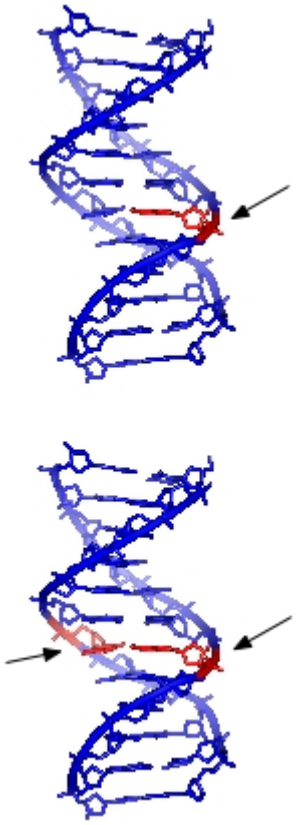
It is important to distinguish between DNA damage and mutation, the two major types of error in DNA. DNA damages and mutation are fundamentally different. Damages are physical abnormalities in the DNA, such as single- and double-strand breaks, 8-hydroxydeoxyguanosine residues, and polycyclic aromatic hydrocarbon adducts. DNA damages can be recognized by enzymes, and, thus, they can be correctly repaired if redundant information, such as the undamaged sequence in the complementary DNA strand or in a homologous chromosome, is available for copying. If a cell retains DNA

damage, transcription of a gene can be prevented, and, thus, translation into a protein will also be blocked. Replication may also be blocked and/or the cell may die.

In contrast to DNA damage, a mutation is a change in the base sequence of the DNA. A mutation cannot be recognized by enzymes once the base change is present in both DNA strands, and, thus, a mutation cannot be repaired. At the cellular level, mutations can cause alterations in protein function and regulation. Mutations are replicated when the cell replicates. In a population of cells, mutant cells will increase or decrease in frequency according to the effects of the mutation on the ability of the cell to survive and reproduce. Although distinctly different from each other, DNA damages and mutations are related because DNA damages often cause errors of DNA synthesis during replication or repair; these errors are a major source of mutation.

Given these properties of DNA damage and mutation, it can be seen that DNA damages are a special problem in non-dividing or slowly dividing cells, where unrepaired damages will tend to accumulate over time. On the other hand, in rapidly dividing cells, unrepaired DNA damages that do not kill the cell by blocking replication will tend to cause replication errors and thus mutation. The great majority of mutations that are not neutral in their effect are deleterious to a cell's survival. Thus, in a population of cells comprising a tissue with replicating cells, mutant cells will tend to be lost. However, infrequent mutations that provide a survival advantage will tend to clonally expand at the expense of neighboring cells in the tissue. This advantage to the cell is disadvantageous to the whole organism, because such mutant cells can give rise to cancer. Thus, DNA damages in frequently dividing cells, because they give rise to mutations, are a prominent cause of cancer. In contrast, DNA damages in infrequently dividing cells are likely a prominent cause of aging.

DNA repair mechanisms



Single-strand and double-strand DNA damage

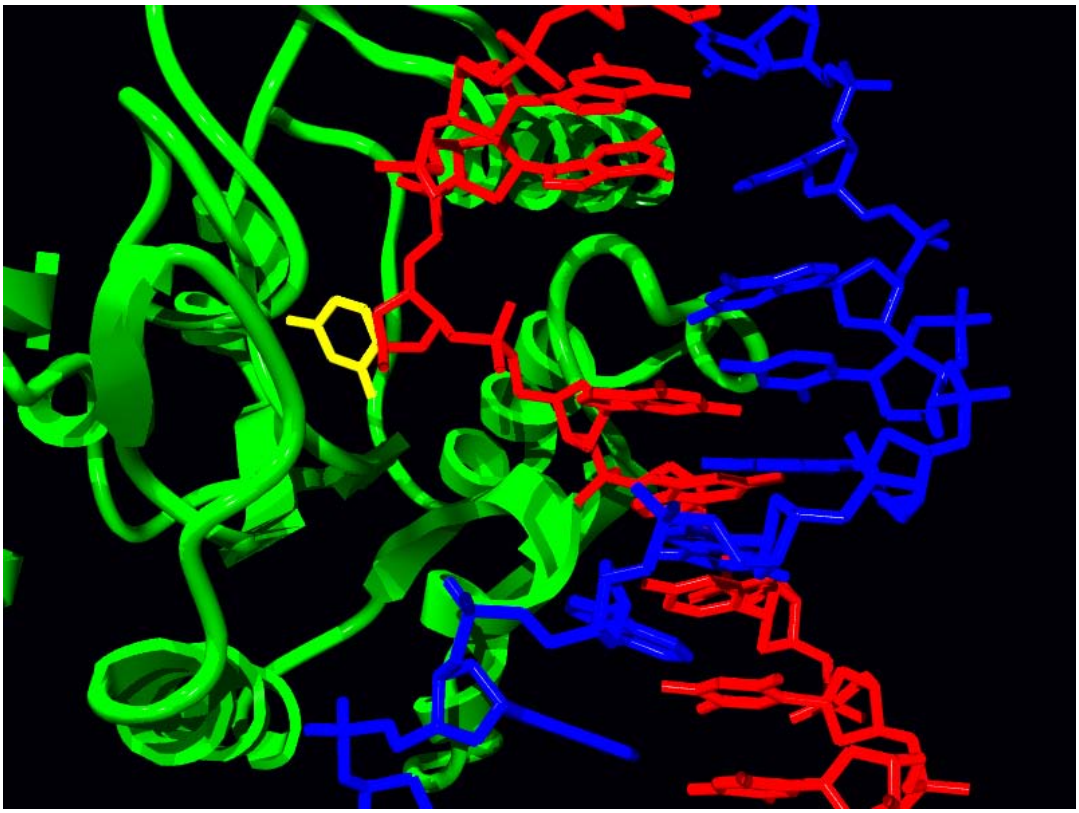
Cells cannot function if DNA damage corrupts the integrity and accessibility of essential information in the genome (but cells remain superficially functional when so-called "non-essential" genes are missing or damaged). Depending on the type of damage inflicted on the DNA's double helical structure, a variety of repair strategies have evolved to restore lost information. If possible, cells use the unmodified complementary strand of the DNA or the sister chromatid as a template to recover the original information. Without access to a template, cells use an error-prone recovery mechanism known as translesion synthesis as a last resort.

Damage to DNA alters the spatial configuration of the helix, and such alterations can be detected by the cell. Once damage is localized, specific DNA repair molecules bind at or near the site of damage, inducing other molecules to bind and form a complex that enables the actual repair to take place. The types of molecules involved and the mechanism of repair that is mobilized depend on the type of damage that has occurred and the phase of the cell cycle that the cell is in.

Direct reversal

Cells are known to eliminate three types of damage to their DNA by chemically reversing it. These mechanisms do not require a template, since the types of damage they counteract can occur in only one of the four bases. Such direct reversal mechanisms are specific to the type of damage incurred and do not involve breakage of the phosphodiester backbone. The formation of pyrimidine dimers upon irradiation with UV light results in an abnormal covalent bond between adjacent pyrimidine bases. The photoreactivation process directly reverses this damage by the action of the enzyme photolyase, whose activation is obligately dependent on energy absorbed from blue/UV light (300–500 nm wavelength) to promote catalysis. Another type of damage, methylation of guanine bases, is directly reversed by the protein methyl guanine methyl transferase (MGMT), the bacterial equivalent of which is called ogt. This is an expensive process because each MGMT molecule can be used only once; that is, the reaction is stoichiometric rather than catalytic. A generalized response to methylating agents in bacteria is known as the adaptive response and confers a level of resistance to alkylating agents upon sustained exposure by upregulation of alkylation repair enzymes. The third type of DNA damage reversed by cells is certain methylation of the bases cytosine and adenine.

Single-strand damage



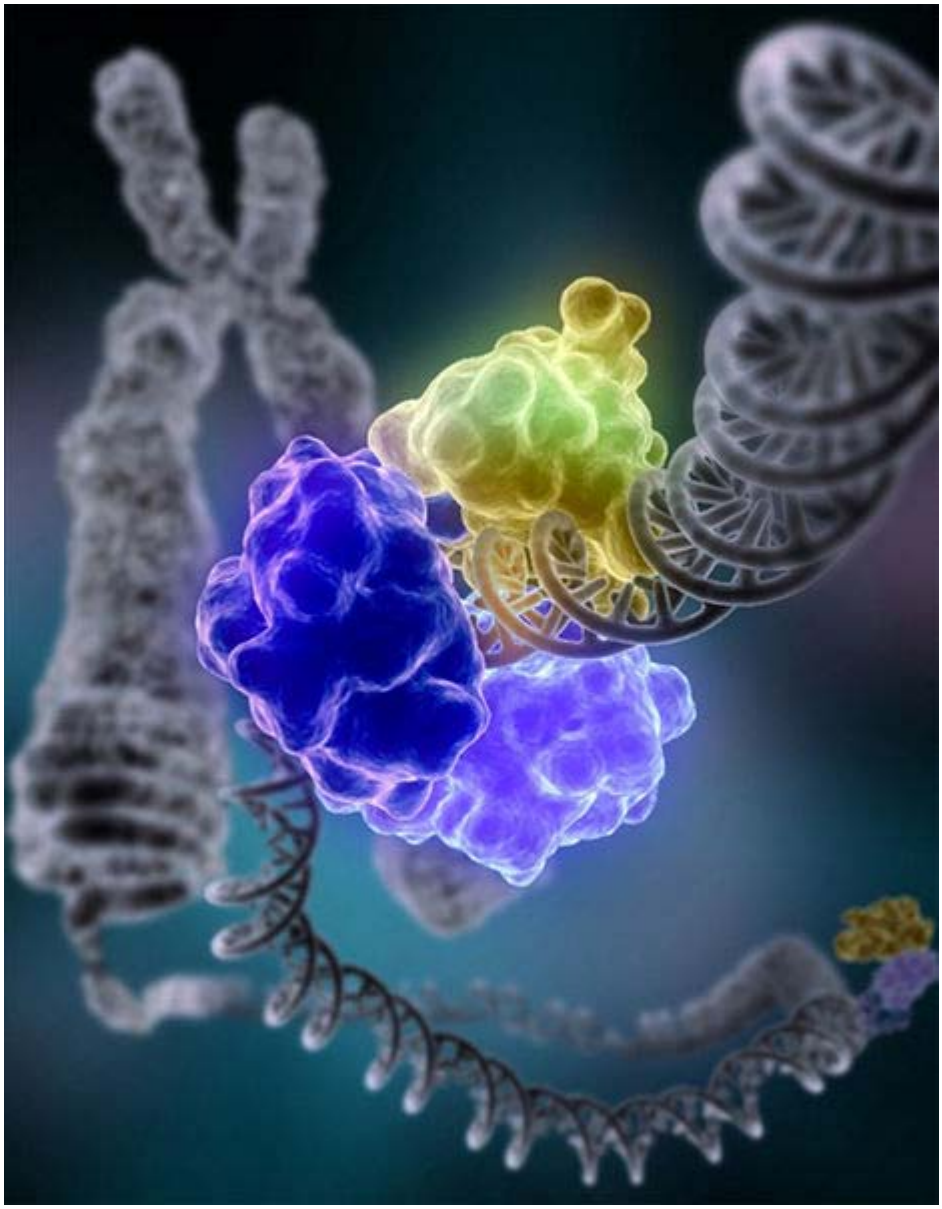
Structure of the base-excision repair enzyme uracil-DNA glycosylase. The uracil residue is shown in yellow.

When only one of the two strands of a double helix has a defect, the other strand can be used as a template to guide the correction of the damaged strand. In order to repair damage to one of the two paired molecules of DNA, there exist a number of excision repair mechanisms that remove the damaged nucleotide and replace it with an undamaged nucleotide complementary to that found in the undamaged DNA strand.

1. Base excision repair (BER), which repairs damage to a single base caused by oxidation, alkylation, hydrolysis, or deamination. The damaged base is removed by a DNA glycosylase. The "missing tooth" is then recognised by an enzyme called AP endonuclease, which cuts the Phosphodiester bond. The missing part is then resynthesized by a DNA polymerase, and a DNA ligase performs the final nick-sealing step.
2. Nucleotide excision repair (NER), which recognizes bulky, helix-distorting lesions such as pyrimidine dimers and 6,4 photoproducts. A specialized form of NER known as transcription-coupled repair deploys NER enzymes to genes that are being actively transcribed.
3. Mismatch repair (MMR), which corrects errors of DNA replication and recombination that result in mispaired (but undamaged) nucleotides.

Double-strand breaks

Double-strand breaks, in which both strands in the double helix are severed, are particularly hazardous to the cell because they can lead to genome rearrangements. Three mechanisms exist to repair double-strand breaks (DSBs): non-homologous end joining (NHEJ), microhomology-mediated end joining (MMEJ), and homologous recombination. PVN Acharya noted that double-strand breaks and a "cross-linkage joining both strands at the same point is irreparable because neither strand can then serve as a template for repair. The cell will die in the next mitosis or in some rare instances, mutate."



DNA ligase, shown above repairing chromosomal damage, is an enzyme that joins broken nucleotides together by catalyzing the formation of an internucleotide ester bond between the phosphate backbone and the deoxyribose nucleotides.

In NHEJ, DNA Ligase IV, a specialized DNA ligase that forms a complex with the cofactor XRCC4, directly joins the two ends. To guide accurate repair, NHEJ relies on short homologous sequences called microhomologies present on the single-stranded tails of the DNA ends to be joined. If these overhangs are compatible, repair is usually accurate. NHEJ can also introduce mutations during repair. Loss of damaged nucleotides at the break site can lead to deletions, and joining of nonmatching termini forms translocations. NHEJ is especially important before the cell has replicated its DNA, since there is no template available for repair by homologous recombination. There are "backup" NHEJ pathways in higher eukaryotes. Besides its role as a genome caretaker,

NHEJ is required for joining hairpin-capped double-strand breaks induced during V(D)J recombination, the process that generates diversity in B-cell and T-cell receptors in the vertebrate immune system.

Homologous recombination requires the presence of an identical or nearly identical sequence to be used as a template for repair of the break. The enzymatic machinery responsible for this repair process is nearly identical to the machinery responsible for chromosomal crossover during meiosis. This pathway allows a damaged chromosome to be repaired using a sister chromatid (available in G2 after DNA replication) or a homologous chromosome as a template. DSBs caused by the replication machinery attempting to synthesize across a single-strand break or unrepaired lesion cause collapse of the replication fork and are typically repaired by recombination.

Topoisomerases introduce both single- and double-strand breaks in the course of changing the DNA's state of supercoiling, which is especially common in regions near an open replication fork. Such breaks are not considered DNA damage because they are a natural intermediate in the topoisomerase biochemical mechanism and are immediately repaired by the enzymes that created them.

A team of French researchers bombarded *Deinococcus radiodurans* to study the mechanism of double-strand break DNA repair in that organism. At least two copies of the genome, with random DNA breaks, can form DNA fragments through annealing. Partially overlapping fragments are then used for synthesis of homologous regions through a moving D-loop that can continue extension until they find complementary partner strands. In the final step there is crossover by means of RecA-dependent homologous recombination.

Translesion synthesis

Translesion synthesis is a DNA damage tolerance process that allows the DNA replication machinery to replicate past DNA lesions such as thymine dimers or AP sites. It involves switching out regular DNA polymerases for specialized translesion polymerases (e.g. DNA polymerase V), often with larger active sites that can facilitate the insertion of bases opposite damaged nucleotides. The polymerase switching is thought to be mediated by, among other factors, the post-translational modification of the replication processivity factor PCNA. Translesion synthesis polymerases often have low fidelity (high propensity to insert wrong bases) relative to regular polymerases. However, many are extremely efficient at inserting correct bases opposite specific types of damage. For example, Pol η mediates error-free bypass of lesions induced by UV irradiation, whereas Pol ζ introduces mutations at these sites. From a cellular perspective, risking the introduction of point mutations during translesion synthesis may be preferable to resorting to more drastic mechanisms of DNA repair, which may cause gross chromosomal aberrations or cell death. In short, the process involves specialized polymerases either bypassing or repairing lesions at locations of stalled DNA replication. A bypass platform is provided to these polymerases by Proliferating cell nuclear antigen (PCNA). Under normal circumstances, PCNA bound to polymerases replicates the DNA.

At a site of lesion, PCNA is ubiquitinated, or modified, by the RAD6/RAD18 proteins to provide a platform for the specialized polymerases to bypass the lesion and resume DNA replication.

Global response to DNA damage

Cells exposed to ionizing radiation, ultraviolet light or chemicals are prone to acquire multiple sites of bulky DNA lesions and double-strand breaks. Moreover, DNA damaging agents can damage other biomolecules such as proteins, carbohydrates, lipids, and RNA. The accumulation of damage, to be specific, double-strand breaks or adducts stalling the replication forks, are among known stimulation signals for a global response to DNA damage. The global response to damage is an act directed toward the cells' own preservation and triggers multiple pathways of macromolecular repair, lesion bypass, tolerance, or apoptosis. The common features of global response are induction of multiple genes, cell cycle arrest, and inhibition of cell division.

DNA damage checkpoints

After DNA damage, cell cycle checkpoints are activated. Checkpoint activation pauses the cell cycle and gives the cell time to repair the damage before continuing to divide. DNA damage checkpoints occur at the G1/S and G2/M boundaries. An intra-S checkpoint also exists. Checkpoint activation is controlled by two master kinases, ATM and ATR. ATM responds to DNA double-strand breaks and disruptions in chromatin structure, whereas ATR primarily responds to stalled replication forks. These kinases phosphorylate downstream targets in a signal transduction cascade, eventually leading to cell cycle arrest. A class of checkpoint mediator proteins including BRCA1, MDC1, and 53BP1 has also been identified. These proteins seem to be required for transmitting the checkpoint activation signal to downstream proteins.

P53 is an important downstream target of ATM and ATR, as it is required for inducing apoptosis following DNA damage. At the G1/S checkpoint, p53 functions by deactivating the CDK2/cyclin E complex. Similarly, p21 mediates the G2/M checkpoint by deactivating the CDK1/cyclin B complex.

The prokaryotic SOS response

The SOS response is the term used to describe changes in gene expression in *Escherichia coli* and other bacteria in response to extensive DNA damage. The prokaryotic SOS system is regulated by two key proteins: LexA and RecA. The LexA homodimer is a transcriptional repressor that binds to operator sequences commonly referred to as SOS boxes. In *Escherichia coli* it is known that LexA regulates transcription of approximately 48 genes including the lexA and recA genes. The SOS response is known to be widespread in the Bacteria domain, but it is mostly absent in some bacterial phyla, like the Spirochetes. The most common cellular signals activating the SOS response are regions of single-stranded DNA (ssDNA), arising from stalled replication forks or double-strand breaks, which are processed by DNA helicase to separate the two DNA

strands. In the initiation step, RecA protein binds to ssDNA in an ATP hydrolysis driven reaction creating RecA–ssDNA filaments. RecA–ssDNA filaments activate LexA autoprotease activity, which ultimately leads to cleavage of LexA dimer and subsequent LexA degradation. The loss of LexA repressor induces transcription of the SOS genes and allows for further signal induction, inhibition of cell division and an increase in levels of proteins responsible for damage processing.

In *Escherichia coli*, SOS boxes are 20-nucleotide long sequences near promoters with palindromic structure and a high degree of sequence conservation. In other classes and phyla, the sequence of SOS boxes varies considerably, with different length and composition, but it is always highly conserved and one of the strongest short signals in the genome. The high information content of SOS boxes permits differential binding of LexA to different promoters and allows for timing of the SOS response. The lesion repair genes are induced at the beginning of SOS response. The error-prone translesion polymerases, for example, UmuCD’2 (also called DNA polymerase V), are induced later on as a last resort. Once the DNA damage is repaired or bypassed using polymerases or through recombination, the amount of single-stranded DNA in cells is decreased, lowering the amounts of RecA filaments decreases cleavage activity of LexA homodimer, which then binds to the SOS boxes near promoters and restores normal gene expression.

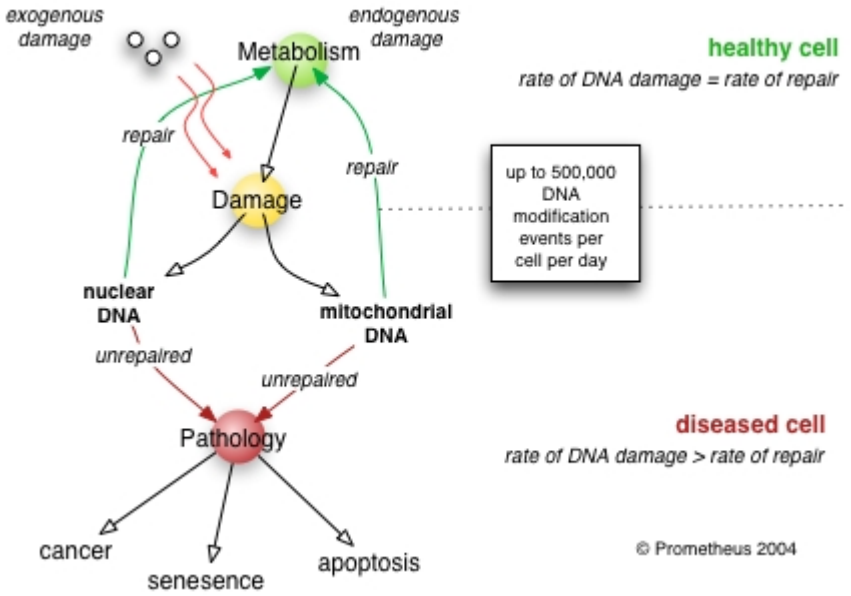
Eukaryotic transcriptional responses to DNA damage

Eukaryotic cells exposed to DNA damaging agents also activate important defensive pathways by inducing multiple proteins involved in DNA repair, cell cycle checkpoint control, protein trafficking and degradation. Such genome wide transcriptional response is very complex and tightly regulated, thus allowing coordinated global response to damage. Exposure of yeast *Saccharomyces cerevisiae* to DNA damaging agents results in overlapping but distinct transcriptional profiles. Similarities to environmental shock response indicates that a general global stress response pathway exist at the level of transcriptional activation. In contrast, different human cell types respond to damage differently indicating an absence of a common global response. The probable explanation for this difference between yeast and human cells may be in the heterogeneity of mammalian cells. In an animal different types of cells are distributed among different organs that have evolved different sensitivities to DNA damage.

In general global response to DNA damage involves expression of multiple genes responsible for postreplication repair, homologous recombination, nucleotide excision repair, DNA damage checkpoint, global transcriptional activation, genes controlling mRNA decay, and many others. A large amount of damage to a cell leaves it with an important decision: undergo apoptosis and die, or survive at the cost of living with a modified genome. An increase in tolerance to damage can lead to an increased rate of survival that will allow a greater accumulation of mutations. Yeast Rev1 and human polymerase η are members of [Y family translesion DNA polymerases present during global response to DNA damage and are responsible for enhanced mutagenesis during a global response to DNA damage in eukaryotes.

DNA repair and aging

Pathological effects of poor DNA repair

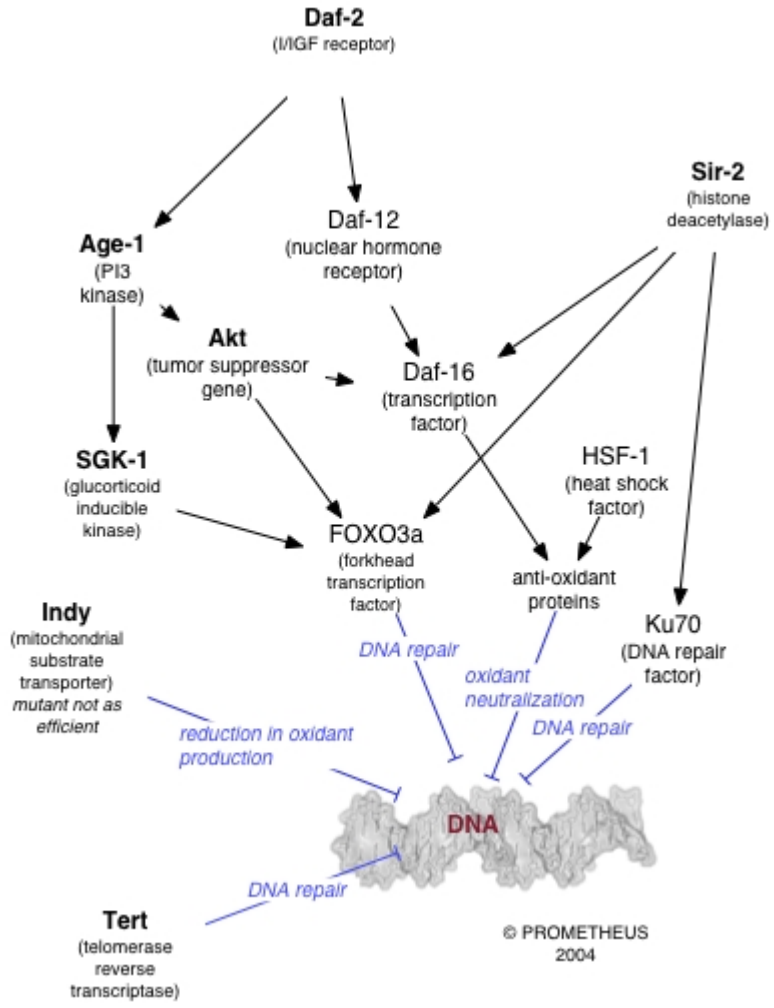


DNA repair rate is an important determinant of cell pathology

Experimental animals with genetic deficiencies in DNA repair often show decreased life span and increased cancer incidence. For example, mice deficient in the dominant NHEJ pathway and in telomere maintenance mechanisms get lymphoma and infections more often, and, as a consequence, have shorter lifespans than wild-type mice. In similar manner, mice deficient in a key repair and transcription protein that unwinds DNA helices have premature onset of aging-related diseases and consequent shortening of lifespan. However, not every DNA repair deficiency creates exactly the predicted effects; mice deficient in the NER pathway exhibited shortened life span without correspondingly higher rates of mutation.

If the rate of DNA damage exceeds the capacity of the cell to repair it, the accumulation of errors can overwhelm the cell and result in early senescence, apoptosis, or cancer. Inherited diseases associated with faulty DNA repair functioning result in premature aging, increased sensitivity to carcinogens, and correspondingly increased cancer risk. On the other hand, organisms with enhanced DNA repair systems, such as *Deinococcus radiodurans*, the most radiation-resistant known organism, exhibit remarkable resistance to the double-strand break-inducing effects of radioactivity, likely due to enhanced efficiency of DNA repair and especially NHEJ.

Longevity and caloric restriction



Most life span influencing genes affect the rate of DNA damage

A number of individual genes have been identified as influencing variations in life span within a population of organisms. The effects of these genes is strongly dependent on the environment, in particular, on the organism's diet. Caloric restriction reproducibly results in extended lifespan in a variety of organisms, likely via nutrient sensing pathways and decreased metabolic rate. The molecular mechanisms by which such restriction results in lengthened lifespan are as yet unclear; however, the behavior of many genes known to be involved in DNA repair is altered under conditions of caloric restriction.

For example, increasing the gene dosage of the gene SIR-2, which regulates DNA packaging in the nematode worm *Caenorhabditis elegans*, can significantly extend lifespan. The mammalian homolog of SIR-2 is known to induce downstream DNA repair factors involved in NHEJ, an activity that is especially promoted under conditions of caloric restriction. Caloric restriction has been closely linked to the rate of base excision

repair in the nuclear DNA of rodents, although similar effects have not been observed in mitochondrial DNA.

It is interesting to note that the *C. elegans* gene AGE-1, an upstream effector of DNA repair pathways, confers dramatically extended life span under free-feeding conditions but leads to a decrease in reproductive fitness under conditions of caloric restriction. This observation supports the pleiotropy theory of the biological origins of aging, which suggests that genes conferring a large survival advantage early in life will be selected for even if they carry a corresponding disadvantage late in life.

Medicine and DNA repair modulation

Hereditary DNA repair disorders

Defects in the NER mechanism are responsible for several genetic disorders, including:

- xeroderma pigmentosum: hypersensitivity to sunlight/UV, resulting in increased skin cancer incidence and premature aging
- Cockayne syndrome: hypersensitivity to UV and chemical agents
- trichothiodystrophy: sensitive skin, brittle hair and nails

Mental retardation often accompanies the latter two disorders, suggesting increased vulnerability of developmental neurons.

Other DNA repair disorders include:

- Werner's syndrome: premature aging and retarded growth
- Bloom's syndrome: sunlight hypersensitivity, high incidence of malignancies (especially leukemias).
- ataxia telangiectasia: sensitivity to ionizing radiation and some chemical agents

All of the above diseases are often called "segmental progerias" ("accelerated aging diseases") because their victims appear elderly and suffer from aging-related diseases at an abnormally young age, while not manifesting all the symptoms of old age.

Other diseases associated with reduced DNA repair function include Fanconi's anemia, hereditary breast cancer and hereditary colon cancer.

DNA repair and cancer

Inherited mutations that affect DNA repair genes are strongly associated with high cancer risks in humans. Hereditary nonpolyposis colorectal cancer (HNPCC) is strongly associated with specific mutations in the DNA mismatch repair pathway. BRCA1 and BRCA2, two famous mutations conferring a hugely increased risk of breast cancer on carriers, are both associated with a large number of DNA repair pathways, especially NHEJ and homologous recombination.

Cancer therapy procedures such as chemotherapy and radiotherapy work by overwhelming the capacity of the cell to repair DNA damage, resulting in cell death. Cells that are most rapidly dividing - most typically cancer cells - are preferentially affected. The side-effect is that other non-cancerous but rapidly dividing cells such as stem cells in the bone marrow are also affected. Modern cancer treatments attempt to localize the DNA damage to cells and tissues only associated with cancer, either by physical means (concentrating the therapeutic agent in the region of the tumor) or by biochemical means (exploiting a feature unique to cancer cells in the body).

DNA repair and evolution

The basic processes of DNA repair are highly conserved among both prokaryotes and eukaryotes and even among bacteriophage (viruses that infect bacteria); however, more complex organisms with more complex genomes have correspondingly more complex repair mechanisms. The ability of a large number of protein structural motifs to catalyze relevant chemical reactions has played a significant role in the elaboration of repair mechanisms during evolution. For an extremely detailed review of hypotheses relating to the evolution of DNA repair, see.

The fossil record indicates that single-cell life began to proliferate on the planet at some point during the Precambrian period, although exactly when recognizably modern life first emerged is unclear. Nucleic acids became the sole and universal means of encoding genetic information, requiring DNA repair mechanisms that in their basic form have been inherited by all extant life forms from their common ancestor. The emergence of Earth's oxygen-rich atmosphere (known as the "oxygen catastrophe") due to photosynthetic organisms, as well as the presence of potentially damaging free radicals in the cell due to oxidative phosphorylation, necessitated the evolution of DNA repair mechanisms that act specifically to counter the types of damage induced by oxidative stress.

Rate of evolutionary change

On some occasions, DNA damage is not repaired, or is repaired by an error-prone mechanism that results in a change from the original sequence. When this occurs, mutations may propagate into the genomes of the cell's progeny. Should such an event occur in a germ line cell that will eventually produce a gamete, the mutation has the potential to be passed on to the organism's offspring. The rate of evolution in a particular species (or, in a particular gene) is a function of the rate of mutation. As a consequence, the rate and accuracy of DNA repair mechanisms have an influence over the process of evolutionary change.

Chapter- 11

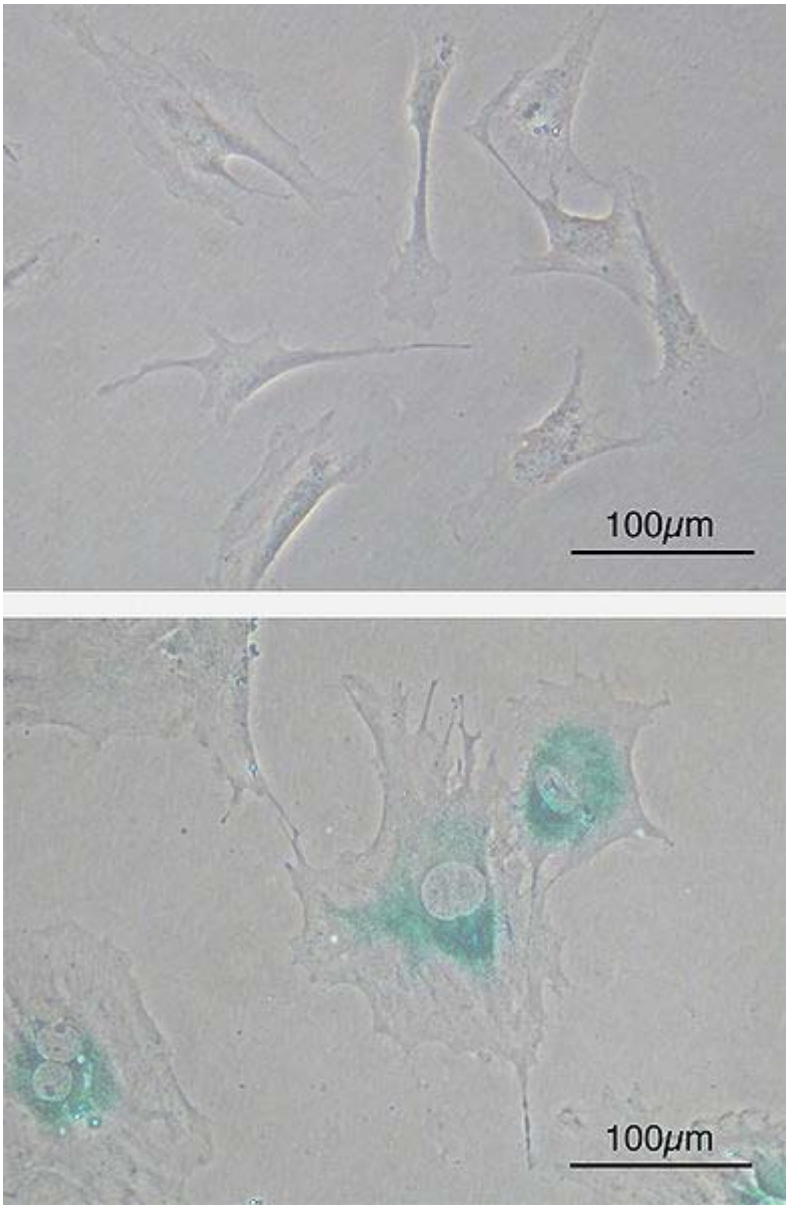
Senescence and Cell Adhesion

Senescence

Senescence or **biological aging** is the change in the biology of an organism as it ages after its maturity. Such changes range from those affecting its cells and their function to that of the whole organism. There are a number of theories as to why senescence occurs, including ones that claim it is programmed by gene expression changes and that it is the accumulative damage of biological processes.

The word *senescence* is derived from the Latin word *senex*, meaning *old man*, *old age*, or *advanced in age*.

Cellular senescence



Cellular senescence
(upper) Primary mouse embryonic fibroblast cells (MEFs) before senescence. Spindle-shaped. (lower) MEFs became senescent after passages. Cells grow larger, flatten shape and expressed senescence-associated β-galactosidase (SABG, blue areas), a marker of cellular senescence.

Cellular senescence is the phenomenon by which normal diploid cells lose the ability to divide, normally after about 50 cell divisions in vitro. Some cells become senescent after fewer replications cycles as a result of DNA double strand breaks, toxins, etc. This phenomenon is also known as "replicative senescence", the "Hayflick phenomenon", or the Hayflick limit in honour of Dr. Leonard Hayflick who was the first to publish this

information in 1965. In response to DNA damage (including shortened telomeres), cells either age or self-destruct (apoptosis, programmed cell death) if the damage cannot be repaired. In this 'cellular suicide', the death of one cell, or more, may benefit the organism as a whole. For example, in plants the death of the water-conducting xylem cells (tracheids and vessel elements) allows the cells to function more efficiently and so deliver water to the upper parts of a plant.

Aging of the whole organism

Organismal senescence is the aging of whole organisms. In general, aging is characterized by the declining ability to respond to stress, increased homeostatic imbalance, and increased risk of aging-associated diseases. Death is the ultimate consequence of aging, though "old age" is not a scientifically recognized cause of death because there is always a specific proximal cause, such as cancer, heart disease, or liver failure.

Differences in maximum life span among species correspond to different "rates of aging". For example, inherited differences in the rate of aging make a mouse elderly at 3 years and a human elderly at 80 years. These genetic differences affect a variety of physiological processes, including the efficiency of DNA repair, antioxidant enzymes, and rates of free radical production.

Supercentenarian Ann Pouder (8 April 1807 – 10 July 1917) photographed on her 110th birthday. A heavily lined face is common in human senescence.

Senescence of the organism gives rise to the Gompertz–Makeham law of mortality, which says that mortality rate rises rapidly with age.

Some animals, such as some reptiles and fish, age slowly (negligible senescence) and exhibit very long lifespans. Some even exhibit "negative senescence", in which mortality falls with age, in disagreement with the Gompertz–Makeham "law".

Whether replicative senescence (Hayflick limit) plays a causative role in organismal aging is at present an active area of investigation.

Theories of aging

The process of senescence is complex, and may derive from a variety of different mechanisms and exist for a variety of different reasons. However, senescence is not universal, and scientific evidence suggests that cellular senescence evolved in certain species because it prevents the onset of cancer. In a few simple species, such as Hydra, senescence is negligible and cannot be detected. All such species have no "post-mitotic" cells; they reduce the effect of damaging free radicals by cell division and dilution. Such species are not immortal, however, as they will eventually fall prey to trauma or disease. Moreover, average lifespans can vary greatly within and between species. This suggests that both genetic and environmental factors contribute to aging.

In general, theories that explain senescence have been divided between the programmed and stochastic theories of aging. Programmed theories imply that aging is regulated by biological clocks operating throughout the lifespan. This regulation would depend on changes in gene expression that affect the systems responsible for maintenance, repair, and defense responses. Stochastic theories blame environmental impacts on living organisms that induce cumulative damage at various levels as the cause of aging, examples of which ranging from damage to DNA, damage to tissues and cells by oxygen radicals (widely known as free radicals countered by the even more well-known antioxidants), and cross-linking.

However, aging is seen as a progressive failure of homeodynamics (homeostasis) involving genes for the maintenance and repair, stochastic events leading to molecular damage and molecular heterogeneity, and chance events determining the probability of death. Since complex and interacting systems of maintenance and repair comprise the homeodynamic (old term: homeostasis) space of a biological system, aging is considered to be a progressive shrinkage of homeodynamic space mainly due to increased molecular heterogeneity.

Evolutionary theories

A gene can be expressed at various life-stages. Therefore, natural selection can support lethal and harmful alleles, if their expression occurs after reproduction. Senescence may be the product of such selection. In addition, aging is believed to have evolved because of the increasingly smaller probability of an organism still being alive at older age, due to

predation and accidents, both of which may be random and age-invariant. It is thought that strategies that result in a higher reproductive rate at a young age, but shorter overall lifespan, result in a higher lifetime reproductive success and are therefore favoured by natural selection. In essence, aging is, therefore, the result of investing resources in reproduction, rather than maintenance of the body (the "Disposable Soma" theory), in light of the fact that accidents, predation, and disease will eventually kill the organism no matter how much energy is devoted to repair of the body. Various other theories of aging exist, and are not necessarily mutually exclusive.

The geneticist J. B. S. Haldane wondered why the dominant mutation that causes Huntington's disease remained in the population, and why natural selection had not eliminated it. The onset of this neurological disease is (on average) at age 45 and is invariably fatal within 10–20 years. Haldane assumed that, in human prehistory, few survived until age 45. Since few were alive at older ages and their contribution to the next generation was therefore small relative to the large cohorts of younger age groups, the force of selection against such late-acting deleterious mutations was correspondingly small. However, if a mutation affected younger individuals, selection against it would be strong. Therefore, late-acting deleterious mutations could accumulate in populations over evolutionary time through genetic drift. This principle has been demonstrated experimentally. And it is these later-acting deleterious mutations that are believed to cause—even allow—age-related mortality.

Peter Medawar formalised this observation in his mutation accumulation theory of aging. "The force of natural selection weakens with increasing age—even in a theoretically immortal population, provided only that it is exposed to real hazards of mortality. If a genetic disaster… happens late enough in individual life, its consequences may be completely unimportant". The 'real hazards of mortality' are, in typical circumstances, predation, disease, and accidents. So, even an immortal population, whose fertility does not decline with time, will have fewer individuals alive in older age groups. This is called 'extrinsic mortality'. Young cohorts, not depleted in numbers yet by extrinsic mortality, contribute far more to the next generation than the few remaining older cohorts, so the force of selection against late-acting deleterious mutations, which affect only these few older individuals, is very weak. The mutations may not be selected against, therefore, and may spread over evolutionary time into the population.

The major testable prediction made by this model is that species that have high extrinsic mortality in nature will age more quickly and have shorter intrinsic lifespans. This is borne out among mammals, the best-studied in terms of life history. There is a correlation among mammals between body size and lifespan, such that larger species live longer than smaller species in controlled/optimum conditions, but there are notable exceptions. For instance, many bats and rodents are of similar size, yet bats live much longer. For instance, the little brown bat, half the size of a mouse, can live 30 years in the wild. A mouse will live 2–3 years even with optimum conditions. The explanation is that bats have fewer predators, so therefore low extrinsic mortality. Thus, more individuals survive to later ages, so the force of selection against late-acting deleterious mutations is stronger. Fewer late-acting deleterious mutations = slower aging = longer lifespan. Birds are also

warm-blooded and are of similar size to many small mammals, yet live often 5–10 times as long. They have fewer predation pressures compared with ground-dwelling mammals. And seabirds, which, in general, have the fewest predators of all birds, live longest.

Also, when examining the body-size vs. lifespan relationship, one will observe that predator mammals tend to have longer lifespans than prey animals in a controlled environment such as a zoo or nature reserve. The explanation for the long lifespans of primates (such as humans, monkeys, and apes) relative to body size is that their intelligence and often sociality helps them avoid becoming prey. Being a predator, being smart, and working together all reduce extrinsic mortality.

Another evolutionary theory of aging was proposed by George C. Williams and involves antagonistic pleiotropy. A single gene may affect multiple traits. Some traits that increase fitness early in life may also have negative effects later in life. But, because many more individuals are alive at young ages than at old ages, even small positive effects early can be strongly selected for, and large negative effects later may be very weakly selected against. Williams suggested the following example: Perhaps a gene codes for calcium deposition in bones, which promotes juvenile survival and will therefore be favored by natural selection; however, this same gene promotes calcium deposition in the arteries, causing negative effects in old age. Therefore, negative effects in old age may reflect the result of natural selection for pleiotropic genes that are beneficial early in life. In this case, fitness is relatively high when Fisher's reproductive value is high and relatively low when Fisher's reproductive value is low.

Gene regulation

A number of genetic components of aging have been identified using model organisms, ranging from the simple budding yeast *Saccharomyces cerevisiae* to worms such as *Caenorhabditis elegans* and fruit flies (*Drosophila melanogaster*). Study of these organisms has revealed the presence of at least two conserved aging pathways.

One of these pathways involves the gene *Sir2*, a NAD+-dependent histone deacetylase. In yeast, Sir2 is required for genomic silencing at three loci: The yeast mating loci, the telomeres and the ribosomal DNA (rDNA). In some species of yeast, replicative aging may be partially caused by homologous recombination between rDNA repeats; excision of rDNA repeats results in the formation of extrachromosomal rDNA circles (ERCs). These ERCs replicate and preferentially segregate to the mother cell during cell division, and are believed to result in cellular senescence by titrating away (competing for) essential nuclear factors. ERCs have not been observed in other species (nor even all strains of the same yeast species) of yeast (which also display replicative senescence), and ERCs are not believed to contribute to aging in higher organisms such as humans (they have not been shown to accumulate in mammals in a similar manner to yeast). Extrachromosomal circular DNA (eccDNA) has been found in worms, flies, and humans. The origin and role of eccDNA in aging, if any, is unknown.

Despite the lack of a connection between circular DNA and aging in higher organisms, extra copies of Sir2 are capable of extending the lifespan of both worms and flies (though, in flies, this finding has not been replicated by other investigators, and the activator of Sir2 resveratrol does not reproducibly increase lifespan in either species). Whether the Sir2 homologues in higher organisms have any role in lifespan is unclear, but the human SIRT1 protein has been demonstrated to deacetylate p53, Ku70, and the forkhead family of transcription factors. SIRT1 can also regulate acetylates such as CBP/p300, and has been shown to deacetylate specific histone residues.

RAS1 and RAS2 also affect aging in yeast and have a human homologue. RAS2 overexpression has been shown to extend lifespan in yeast.

Other genes regulate aging in yeast by increasing the resistance to oxidative stress. Superoxide dismutase, a protein that protects against the effects of mitochondrial free radicals, can extend yeast lifespan in stationary phase when overexpressed.

In higher organisms, aging is likely to be regulated in part through the insulin/IGF-1 pathway. Mutations that affect insulin-like signaling in worms, flies, and the growth hormone/IGF1 axis in mice are associated with extended lifespan. In yeast, Sir2 activity is regulated by the nicotinamidase PNC1. PNC1 is transcriptionally upregulated under stressful conditions such as caloric restriction, heat shock, and osmotic shock. By converting nicotinamide to niacin, nicotinamide is removed, inhibiting the activity of Sir2. A nicotinamidase found in humans, known as PBEF, may serve a similar function, and a secreted form of PBEF known as visfatin may help to regulate serum insulin levels. It is not known, however, whether these mechanisms also exist in humans, since there are obvious differences in biology between humans and model organisms.

Sir2 activity has been shown to increase under calorie restriction. Due to the lack of available glucose in the cells, more NAD+ is available and can activate Sir2. Resveratrol, a stilbenoid found in the skin of red grapes, was reported to extend the lifespan of yeast, worms, and flies (the lifespan extension in flies and worms have proved irreproducible by independent investigators). It has been shown to activate Sir2 and therefore mimics the effects of calorie restriction, if one accepts that caloric restriction is indeed dependent on Sir2.

Gene expression is imperfectly controlled, and it is possible that random fluctuations in the expression levels of many genes contribute to the aging process as suggested by a study of such genes in yeast. Individual cells, which are genetically identical, none-the-less can have substantially different responses to outside stimuli, and markedly different lifespans, indicating the epigenetic factors play an important role in gene expression and aging as well as genetic factors.

The following is a list of genes connected to longevity through research on model organisms: the filamentous fungus (*Podospora anserina*), bakers' yeast (*Saccharomyces cerevisiae*), the soil roundworm (*Caenorhabditis elegans*), the fruit fly (*Drosophila melanogaster*), and the mouse (*Mus musculus*).

Podospora	Saccharomyces	Caenorhabditis	Drosophila	Mus
grisea	LAG1	daf-2	sod1	Prop-1
	LAC1	age-1/daf-23	cat1	p66shc (Not independently verified)
	pit-1	Ghr		
	RAS1	daf-18	mth	mclk1
	RAS2	akt-1/akt-2		
	PHB1	daf-16		
	PHB2	daf-12		
	CDC7	ctl-1		
	BUD1	old-1		
	RTG2	spe-26		
	RPD3	clk-1		
	HDA1	mev-1		
	SIR2			
		aak-2		
	SIR4-42			
	UTH4			
	YGL023			
	SGS1			
	RAD52			
	FOB1			

Cellular senescence

As noted above, senescence is not universal, and senescence is not observed in single-celled organisms that reproduce through the process of cellular mitosis. Moreover, cellular senescence is not observed in several organisms, including perennial plants, sponges, corals, and lobsters. In those species where cellular senescence is observed, cells eventually become post-mitotic when they can no longer replicate themselves through the process of cellular mitosis; i.e., cells experience *replicative senescence*. How and why some cells become post-mitotic in some species has been the subject of much research and speculation, but (as noted above) it is widely believed that cellular senescence evolved as a way to prevent the onset and spread of cancer. Somatic cells that have divided many times will have accumulated DNA mutations and would therefore be in danger of becoming cancerous if cell division continued.

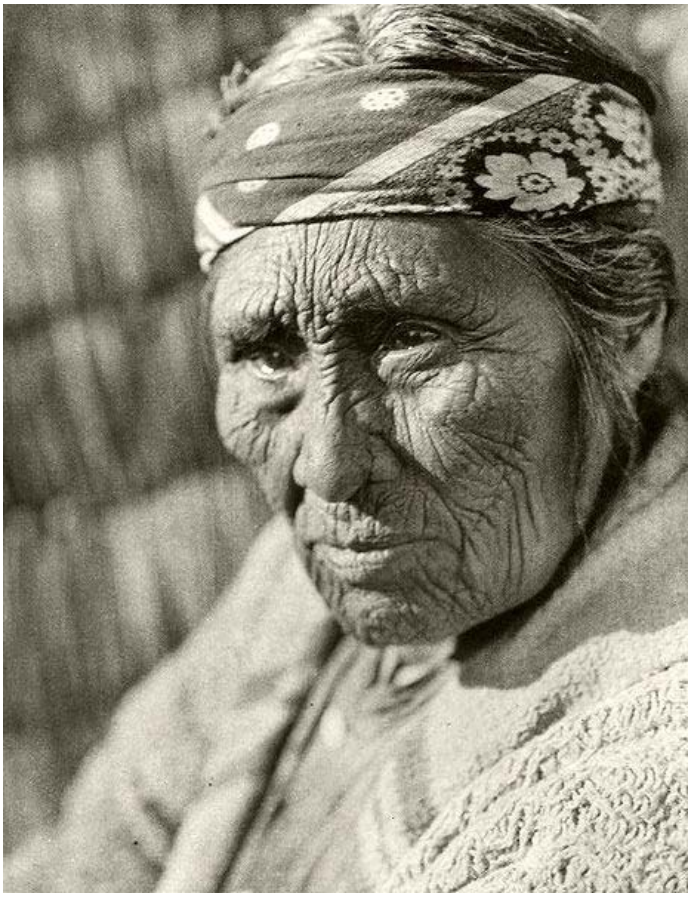
Lately, the role of telomeres in cellular senescence has aroused general interest, especially with a view to the possible genetically adverse effects of cloning. The successive shortening of the chromosomal telomeres with each cell cycle is also believed to limit the number of divisions of the cell, thus contributing to aging. There have, on the other hand, also been reports that cloning could alter the shortening of telomeres. Some

cells do not age and are, therefore, described as being "biologically immortal". It is theorized by some that when it is discovered exactly what allows these cells, whether it be the result of telomere lengthening or not, to divide without limit that it will be possible to genetically alter other cells to have the same capability. It is further theorized that it will eventually be possible to genetically engineer all cells in the human body to have this capability by employing gene therapy and, therefore, stop or reverse aging, effectively making the entire organism potentially immortal.

Cancer cells are usually immortal. In about 85% of tumors, this evasion of cellular senescence is the result of up-activation of their telomerase genes. This simple observation suggests that reactivation of telomerase in healthy individuals could greatly increase their cancer risk.

Whether cell senescence plays any role in organismal aging is at present unknown, and is an active area of investigation. Mouse mutants lacking telomerase do not immediately show accelerated aging.

Chemical damage



Elderly Klamath woman photographed by Edward S. Curtis in 1924

One of the earliest aging theories was the *Rate of Living Hypothesis* described by Raymond Pearl in 1928(based on earlier work by Max Rubner), which states that fast basal metabolic rate corresponds to short maximum life span.

While there may be some validity to the idea that for various types of specific damage detailed below that are by-products of metabolism, all other things being equal, a fast metabolism may reduce lifespan, in general this theory does not adequately explain the differences in lifespan either within, or between, species. Calorically-restricted animals process as much, or more, calories per gram of body mass, as their *ad libitum* fed counterparts, yet exhibit substantially longer lifespans. . Similarly, metabolic rate is a poor predictor of lifespan for birds, bats and other species that, it is presumed, have reduced mortality from predation, and therefore have evolved long lifespans even in the presence of very high metabolic rates. More recently, it was shown that, when modern statistical methods for correcting for the effects of body size and phylogeny are employed, metabolic rate does not correlate with longevity in mammals or birds.

With respect to specific types of chemical damage caused by metabolism, it is suggested that damage to long-lived biopolymers, such as structural proteins or DNA, caused by ubiquitous chemical agents in the body such as oxygen and sugars, are in part responsible for aging. The damage can include breakage of biopolymer chains, cross-linking of biopolymers, or chemical attachment of unnatural substituents (haptens) to biopolymers.

Under normal aerobic conditions, approximately 4% of the oxygen metabolized by mitochondria is converted to superoxide ion, which can subsequently be converted to hydrogen peroxide, hydroxyl radical and eventually other reactive species including other peroxides and singlet oxygen, which can, in turn, generate free radicals capable of damaging structural proteins and DNA. Certain metal ions found in the body, such as copper and iron, may participate in the process. (In Wilson's disease, a hereditary defect that causes the body to retain copper, some of the symptoms resemble accelerated senescence.) These processes are termed *oxidative damage* and are linked to the benefits of nutritionally derived polyphenol antioxidants.

Sugars such as glucose and fructose can react with certain amino acids such as lysine and arginine and certain DNA bases such as guanine to produce sugar adducts, in a process called *glycation*. These adducts can further rearrange to form reactive species, which can then cross-link the structural proteins or DNA to similar biopolymers or other biomolecules such as non-structural proteins. People with diabetes, who have elevated blood sugar, develop senescence-associated disorders much earlier than the general population, but can delay such disorders by rigorous control of their blood sugar levels. There is evidence that sugar damage is linked to oxidant damage in a process termed *glycoxidation*.

Free radicals can damage proteins, lipids or DNA. Glycation mainly damages proteins. Damaged proteins and lipids accumulate in lysosomes as lipofuscin. Chemical damage to structural proteins can lead to loss of function; for example, damage to collagen of blood vessel walls can lead to vessel-wall stiffness and, thus, hypertension, and vessel wall

thickening and reactive tissue formation (atherosclerosis); similar processes in the kidney can lead to renal failure. Damage to enzymes reduces cellular functionality. Lipid peroxidation of the inner mitochondrial membrane reduces the electric potential and the ability to generate energy. It is probably no accident that nearly all of the so-called "accelerated aging diseases" are due to defective DNA repair enzymes.

It is believed that the impact of alcohol on aging can be partly explained by alcohol's activation of the HPA axis, which stimulates glucocorticoid secretion, long-term exposure to which produces symptoms of aging.

Reliability theory

Reliability theory suggests that biological systems start their adult life with a high load of initial damage. Reliability theory is a general theory about systems failure. It allows researchers to predict the age-related failure kinetics for a system of given architecture (reliability structure) and given reliability of its components. Reliability theory predicts that even those systems that composed entirely of non-aging elements (with a constant failure rate) will nevertheless deteriorate (fail more often) with age, if these systems are redundant in irreplaceable elements. Aging, therefore, is a direct consequence of systems.

Reliability theory also predicts the late-life mortality deceleration with subsequent leveling-off, as well as the late-life mortality plateaus, as an inevitable consequence of redundancy exhaustion at extreme old ages. The theory explains why mortality rates increase exponentially with age (the Gompertz law) in many species, by taking into account the initial flaws (defects) in newly formed systems. It also explains why organisms "prefer" to die according to the Gompertz law, while technical devices usually fail according to the Weibull (power) law. Reliability theory allows to specify conditions when organisms die according to the Weibull distribution: Organisms should be relatively free of initial flaws and defects. The theory makes it possible to find a general failure law applicable to all adult and extreme old ages, where the Gompertz and the Weibull laws are just special cases of this more general failure law. The theory explains why relative differences in mortality rates of compared populations (within a given species) vanish with age (compensation law of mortality), and mortality convergence is observed due to the exhaustion of initial differences in redundancy levels.

Miscellaneous

Recently, a kind of early senescence has been alleged to be a possible unintended outcome of early cloning experiments. The issue was raised in the case of Dolly the sheep, following her death from a contagious lung disease. The claim that Dolly's early death involved premature senescence has been vigorously contested, and Dolly's creator, Dr. Ian Wilmut has expressed the view that her illness and death were probably unrelated to the fact that she was a clone.

A set of rare hereditary (genetic) disorders, each called progeria, has been known for some time. Sufferers exhibit symptoms resembling accelerated aging, including wrinkled

skin. The cause of Hutchinson–Gilford progeria syndrome was reported in the journal *Nature* in May 2003. This report suggests that DNA damage, not oxidative stress, is the cause of this form of accelerated aging.

Cell adhesion

Cellular adhesion is the binding of a cell to a surface, extracellular matrix or another cell using cell adhesion molecules such as selectins, integrins, and cadherins. Correct cellular adhesion is essential in maintaining multicellular structure. Cellular adhesion can link the cytoplasm of cells and can be involved in signal transduction.

Process

Cell adhesion molecules involved in the process are first hydrolyzed by extracellular enzymes. Cell adhesion is directly related to protein absorption.

Eukaryotes

Eukaryotic protozoans express multiple adhesion molecules. An example of a pathogenic protozoan is the malarial parasite (*Plasmodium falciparum*), which uses one adhesion molecule called the circumsporozoite protein to bind to liver cells, and another adhesion molecule called the merozoite surface protein to bind red blood cells. In human cells, which have many different types of adhesion molecules, the major classes are named integrins, Ig superfamily members, cadherins, and selectins. Each of these adhesion molecules has a different function and recognizes different ligands. Defects in cell adhesion are usually attributable to defects in expression of adhesion molecules.

Cell junctions allow cells to adhere. There are 4 types of cell junctions :

-Anchoring junctions, which transmit stress through tethering to cytoskeleton are cell-cell or cell-matrix.
-Occluding junctions, which seal gaps between cells, making an impermeable barrier.
-Channel forming junctions aka Gap junctions, which links cytoplasm of adjacent cells.
-Signal relaying junction, synapses in nervous systems

Cell-cell adhesions in Anchoring junctions are mediated by cadherins. Cell-matrix adhesions however, are usually mediated by intergrins .

Selective cell-cell adhesion enables vertebrate cells to assemble into organised tissues. homophilic attachment allows selective recognition resulting. Cells of a similar type stick together where as cells of a different type stay segregated .

Prokaryotes

Prokaryotes have adhesion molecules usually termed "adhesins". Adhesins may occur on pili (fimbriae), flagellae, or the cell surface. Adhesion of bacteria is the first step in colonization and regulates tropism (tissue- or cell-specific interactions).

Viruses

Viruses also have adhesion molecules required for viral binding to host cells. For example, influenza virus has a hemagglutinin on its surface that is required for recognition of the sugar sialic acid on host cell surface molecules. HIV has an adhesion molecule termed gp120 that binds to its ligand CD4, which is expressed on lymphocytes.

Differential adhesion hypothesis

The **differential adhesion hypothesis** (sometimes called the "thermodynamic hypothesis") is a theory of cell adhesion advanced by Malcolm Steinberg in 1964 to explain the mechanism by which heterotypic cells in mixed aggregates sort out into isotypic territories. The DAH postulates that tissues are viscoelastic liquids, and as such possess measurable tissue surface tensions. These surface tensions have been determined for a variety of tissues, including embryonic tissues and cell lines. The surface tensions correspond to the mutual sorting behavior: the tissue type with the higher surface tension will occupy an internal position relative to a tissue with a lower surface tension (if these tissues can interact with each other through their adhesion machinery). Quantitative differences in homo and heterotypic adhesion are supposed to be sufficient to account for the phenomenon without the need to postulate cell type specific adhesion systems: fairly generally accepted, although some tissue specific cell adhesion molecules are now known to exist.

Clinical Implications

Dysfunction of cell-adhesion and cell-migration occurs during cancer metastasis. Cellular adhesion and traction can allow cells to migrate. Cells can form intergrin mediated attachments sites called focal adhesions. Focal adhesions at the leading edge provide the necessary traction allowing the cell to pull its self forward.