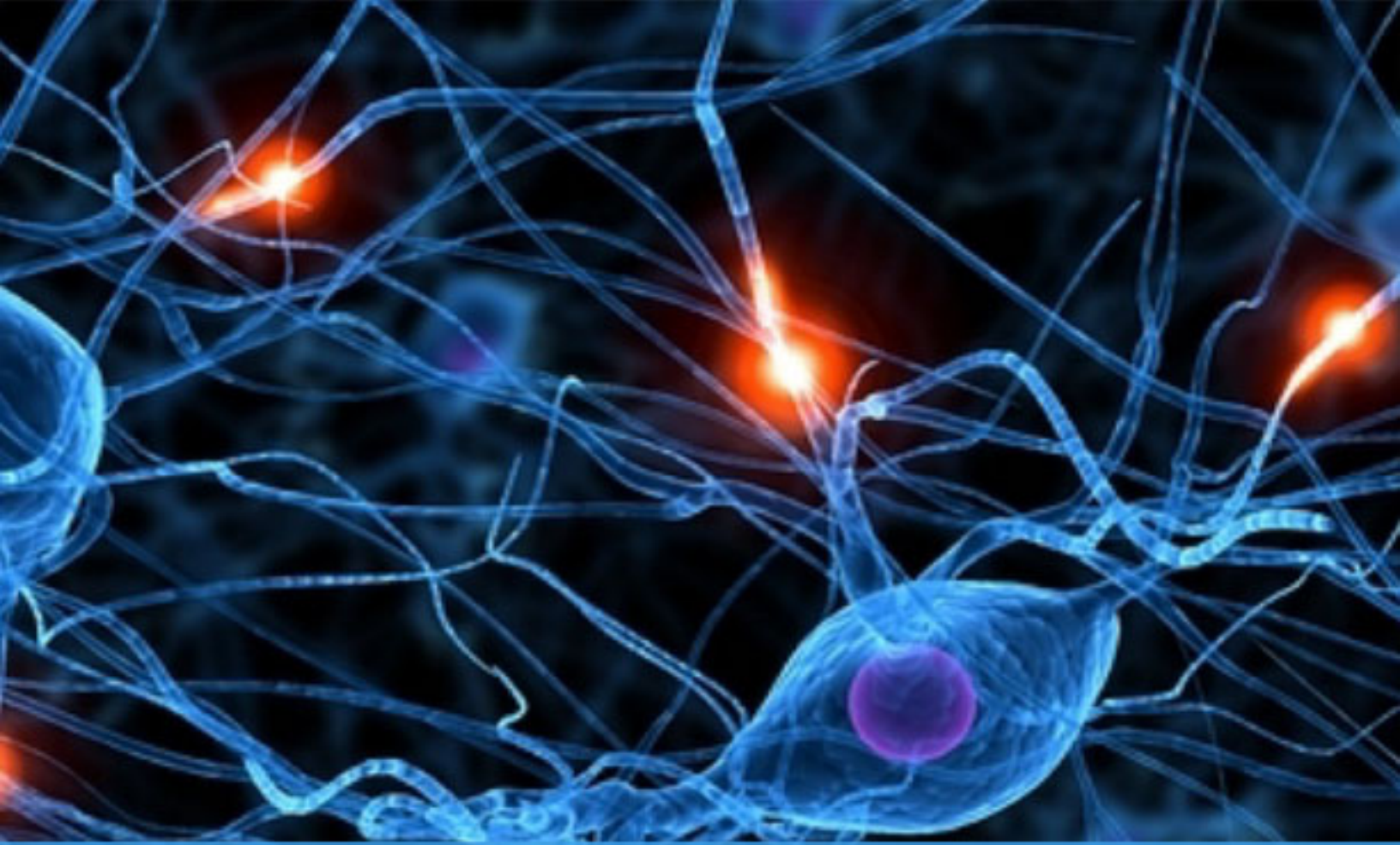

Aspects of cells in Biology

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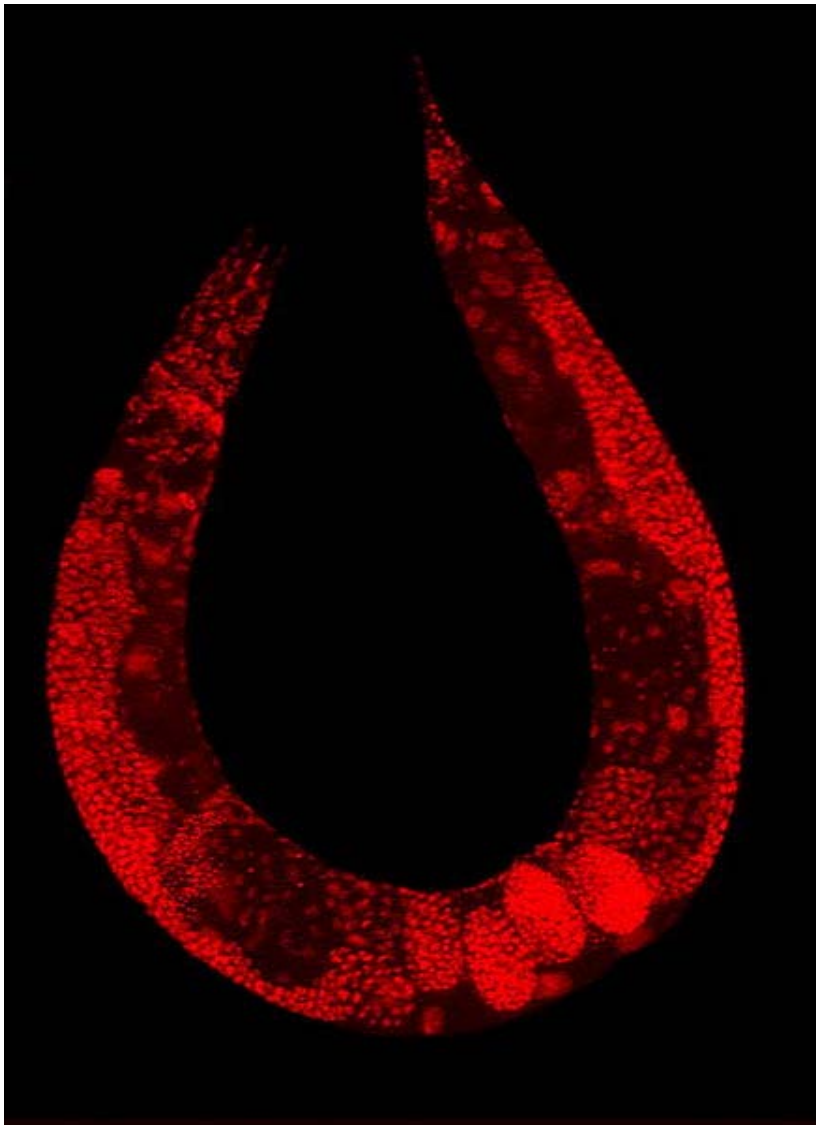
Chapter- 1

Multicellular Organism



In this image, a wild-type *Caenorhabditis elegans* is stained to highlight the nuclei of its cells.

Multicellular organisms are organisms that consist of more than one cell, in contrast to single-celled organisms. Most life that can be seen with the naked eye is multicellular, as are all animals (except for specialized organisms such as Myxozoa) and land plants.

Evolutionary history

Early life was most probably single celled. Multicellularity has evolved independently dozens of times in the history of Earth, for example in plants and animals. Multicellularity exists in both prokaryotes and eukaryotes, and first appeared several billion years ago in cyanobacteria. In order to reproduce, true multicellular organisms must solve the problem of regenerating a whole organism from germ cells (i.e. sperm and egg cells), an issue that is studied in developmental biology. Therefore, the development of sexual reproduction in unicellular organisms during the Mesoproterozoic is thought to have precipitated the development and rise of multicellular life.

Multicellular organisms, especially long-living animals, also face the challenge of cancer, which occurs when cells fail to regulate their growth within the normal program of development. Changes in tissue morphology can be observed during this process.

Hypotheses for origin

There are various mechanisms which are disputed as being the first responsible for the emergence of multicellularity, but it is difficult to say which is correct. This is because all the suggested mechanisms are viable, but establishing which was responsible for the first multicellular life requires mostly speculation.

One hypothesis is that a group of function-specific cells aggregated into a slug-like mass called a grex, which moved as a multicellular unit. Another hypothesis is that a primitive cell underwent nucleus division, thereby becoming a syncytium. A membrane would then form around each nucleus (and the cellular space and organelles occupied in the space), thereby resulting in a group of connected and specialized cells in one organism (this mechanism is observable in Drosophila). A third hypothesis is that, as a unicellular organism divided, the daughter cells failed to separate, resulting in a conglomeration of identical cells in one organism, which could later develop specialized tissues.

Because the first multicellular organisms would have lacked hard body parts, they are not well preserved in fossil records. The earliest fossils of multicellular organisms include the contested Grypania spiralis and the fossils of the black shales of the Palaeoproterozoic Francevillian Group Fossil B Formation in Gabon.

Until recently phylogenetic reconstruction has been through anatomical (particularly embryological) similarities. This is very inexact, as current multicellular organisms such as animals and plants are 500 million years removed from their single celled ancestors. This allows both divergent and convergent evolutionary processes a huge amount of time to mimic similarities and accumulate differences between groups of modern and ancestral

species that no longer exist. While modern phylogenetics uses more sophisticated techniques such as alloenzymes, satellite DNA and other molecular markers, they are still rather imprecise over such huge timescales. Nevertheless, it is hypothesized that the evolution of multicellularity in most, if not all, extant clades could have happened in one of three distinct ways:

The Symbiotic Theory

This theory suggests that the first multicellular organisms occurred from symbiosis (cooperation) of different species of single celled organisms, each with different roles. Over time these organisms would become so dependent on each other they would not be able to survive independently, eventually leading to their genomes being incorporated into one, multicellular, organism. Each respective organism would become a separate lineage of differentiated cells within the newly created species.

This kind of severely co-dependent symbiosis can be seen frequently, such as in the relationship between clown fish and Riterri sea anemones. In these cases it is extremely doubtful if either species would survive very long if the other became extinct. However, the problem with this theory is that it is still not known how each organism's DNA could be incorporated into one single genome to constitute them as a single species. Although such symbiosis is theorized to have occurred (e.g. mitochondria and chloroplasts in animal and plant cells - endosymbiosis) it has only happened extremely rarely and, even then, the genomes of the endosymbionts have retained an element of distinction, separately replicating their DNA during mitosis of the host species. For instance, the two or three symbiotic organisms forming the composite lichen, while dependent on each other for survival, have to separately reproduce and then re-form to create one individual organism once more.

The Cellularization (Syncytial) Theory

This theory states that a single unicellular organism could have developed internal membrane partitions around each of its nuclei Many protists such as the ciliates or slime moulds can have several nuclei, lending support to this hypothesis. However, simple presence of multiple nuclei is not enough to support the theory. Multiple nuclei of ciliates are dissimilar and have clear differentiated functions: the macronucleus serves the organism's needs while the micronucleus is used for sexual-like reproduction with exchange of genetic material. Slime molds syncitia form from individual amoeboid cells, like syncitial tissues of some multicellular organisms, not the other way round. To be deemed valid, this theory needs a demonstrable example and mechanism of generation of a multicellular organism from a pre-existing syncytium.

The Colonial Theory

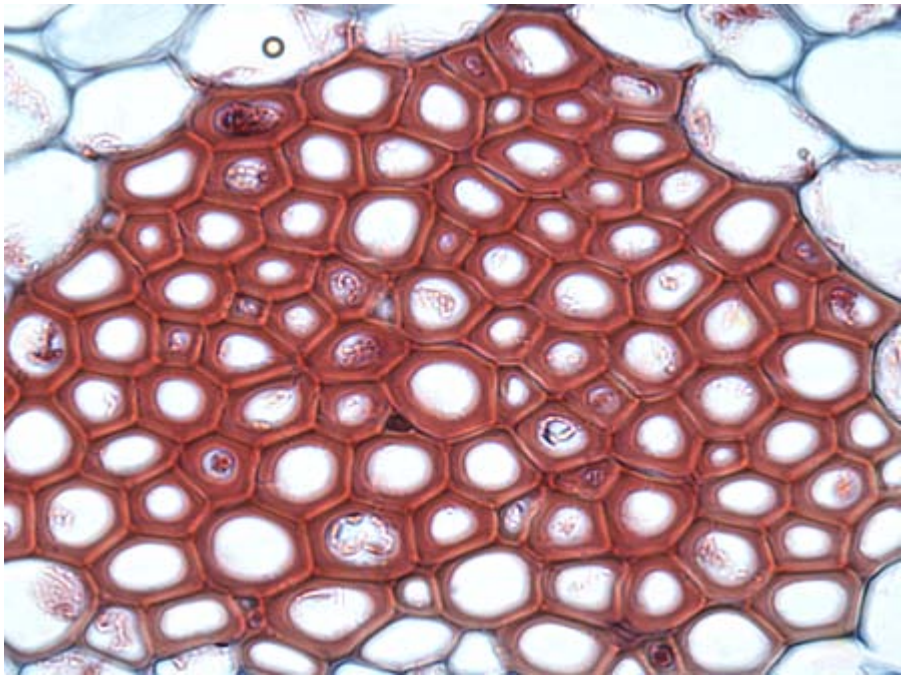
The third explanation of multicellularisation is the Colonial Theory proposed by Haeckel in 1874. This theory claims that the symbiosis of many organisms of the same species (unlike the symbiotic theory, which suggests the symbiosis of different species) led to a

multicellular organism. At least some, presumably land-evolved, multicellularity occurs by cells separating and then rejoining (e.g., cellular slime molds) whereas for the majority of multicellular types (those that evolved within aquatic environments), multicellularity occurs as a consequence of cells failing to separate following division. The mechanism of this latter colony formation can be as simple as incomplete cytokinesis, though multicellularity is also typically considered to involve cellular differentiation

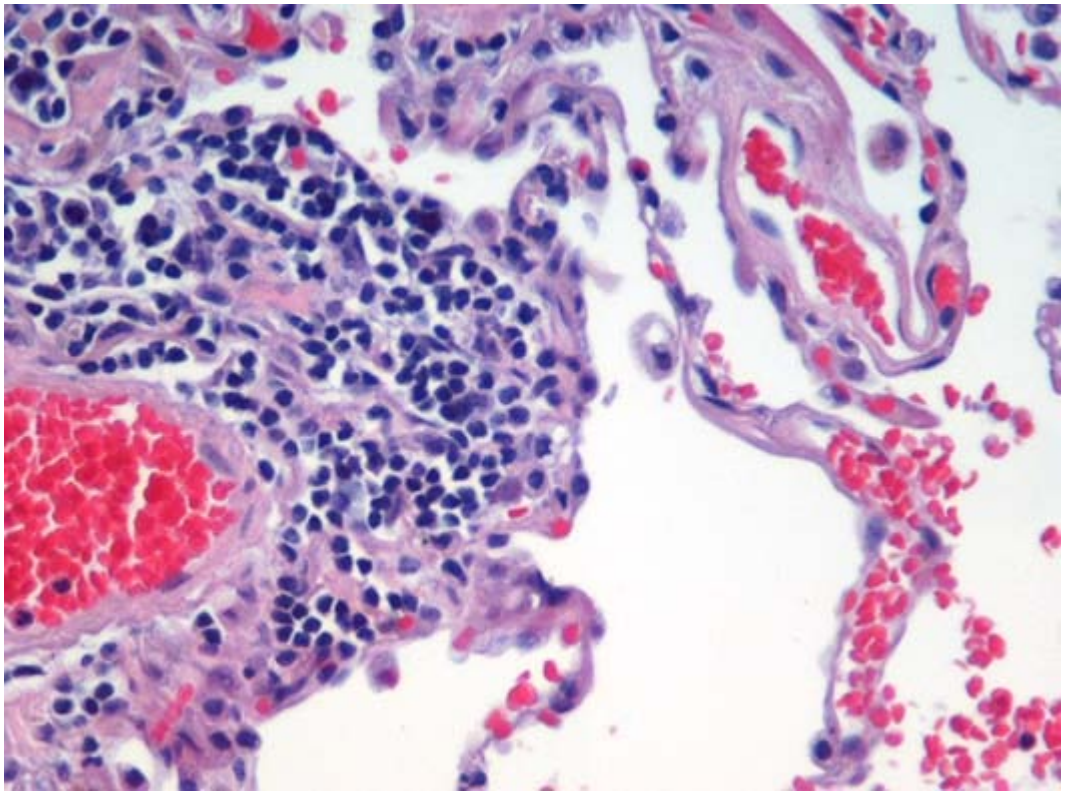
The advantage of the Colonial Theory hypothesis is that it has been seen to occur independently numerous times (in 16 different protoctistan phyla). For instance, during food shortages the amoeba Dictyostelium groups together in a colony that moves as one to a new location. Some of these amoeba then slightly differentiate from each other. Other examples of colonial organisation in protozoa are Volvocaceae, such as Eudorina and Volvox (the latter of which consists of up to 500 — 50,000 cells (depending on the species), only a fraction of which reproduce (in one species 25 — 35, 8 asexually and around 15 — 25 sexually). However, it can often be hard to separate colonial protists from true multicellular organisms, as the two concepts are not distinct. This problem plagues most hypotheses of how multicellularisation could have occurred.

Chapter- 2

Tissue (Biology)



Cross section of sclerenchyma fibers in plant ground tissue

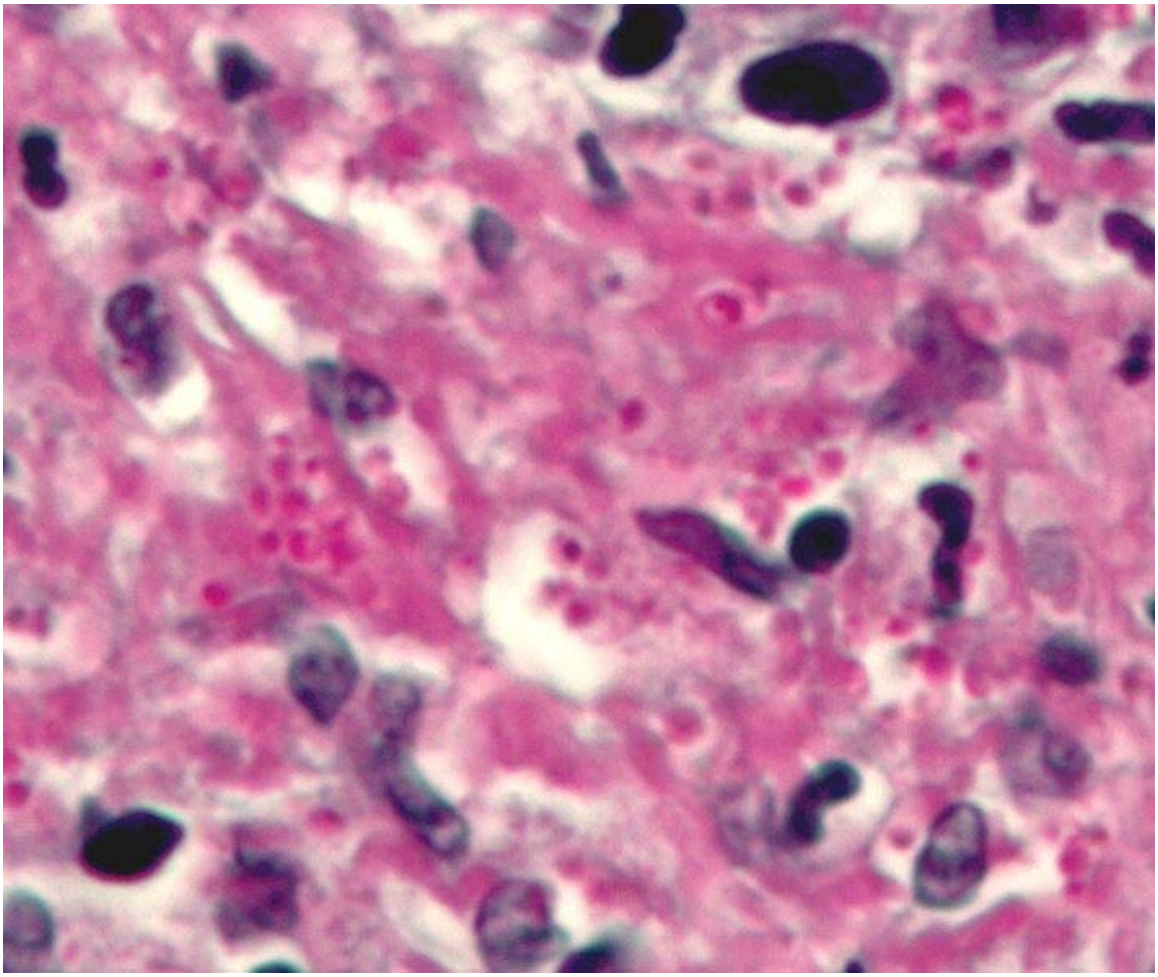


Microscopic view of a histologic specimen of human lung tissue stained with hematoxylin and eosin.

Tissue is a cellular organizational level intermediate between cells and a complete organism. A tissue is an ensemble of cells, not necessarily identical, but from the same origin, that together carry out a specific function. **Organs** are then formed by the functional grouping together of multiple tissues.

The study of tissue is known as histology or, in connection with disease, histopathology. The classical tools for studying tissues are the paraffin block in which tissue is embedded and then sectioned, the histological stain, and the optical microscope. In the last couple of decades, developments in electron microscopy, immunofluorescence, and the use of frozen tissue sections have enhanced the detail that can be observed in tissues. With these tools, the classical appearances of tissues can be examined in health and disease, enabling considerable refinement of clinical diagnosis and prognosis.

Animal tissues



PAS diastase showing the fungus Histoplasma.

Animal tissues can be grouped into four basic types: connective, muscle, nervous, and epithelial. Multiple tissue types comprise organs and body structures. While all animals can generally be considered to contain the four tissue types, the manifestation of these tissues can differ depending on the type of organism. For example, the origin of the cells comprising a particular tissue type may differ developmentally for different classifications of animals. The epithelium in all animals is derived from the ectoderm and endoderm with a small contribution from the mesoderm which forms the endothelium. By contrast, a true epithelial tissue is present only in a single layer of cells held together via occluding junctions called tight junctions, to create a selectively permeable barrier. This tissue covers all organismal surfaces that come in contact with the external environment such as the skin, the airways, and the digestive tract. It serves functions of protection, secretion, and absorption, and is separated from other tissues below by a basal lamina. Endothelium, which comprises the vasculature, is a specialized type of epithelium.

Connective tissue

Connective tissues are fibrous tissues. They are made up of cells separated by non-living material, which is called extracellular matrix. Connective tissue gives shape to organs and holds them in place.

Muscle tissue

Muscle cells form the active contractile tissue of the body known as muscle tissue. Muscle tissue functions to produce force and cause motion, either locomotion or movement within internal organs. Muscle tissue is separated into three distinct categories: visceral or smooth muscle, which is found in the inner linings of organs; skeletal muscle, in which is found attached to bone providing for gross movement; and cardiac muscle which is found in the heart, allowing it to contract and pump blood throughout an organism.

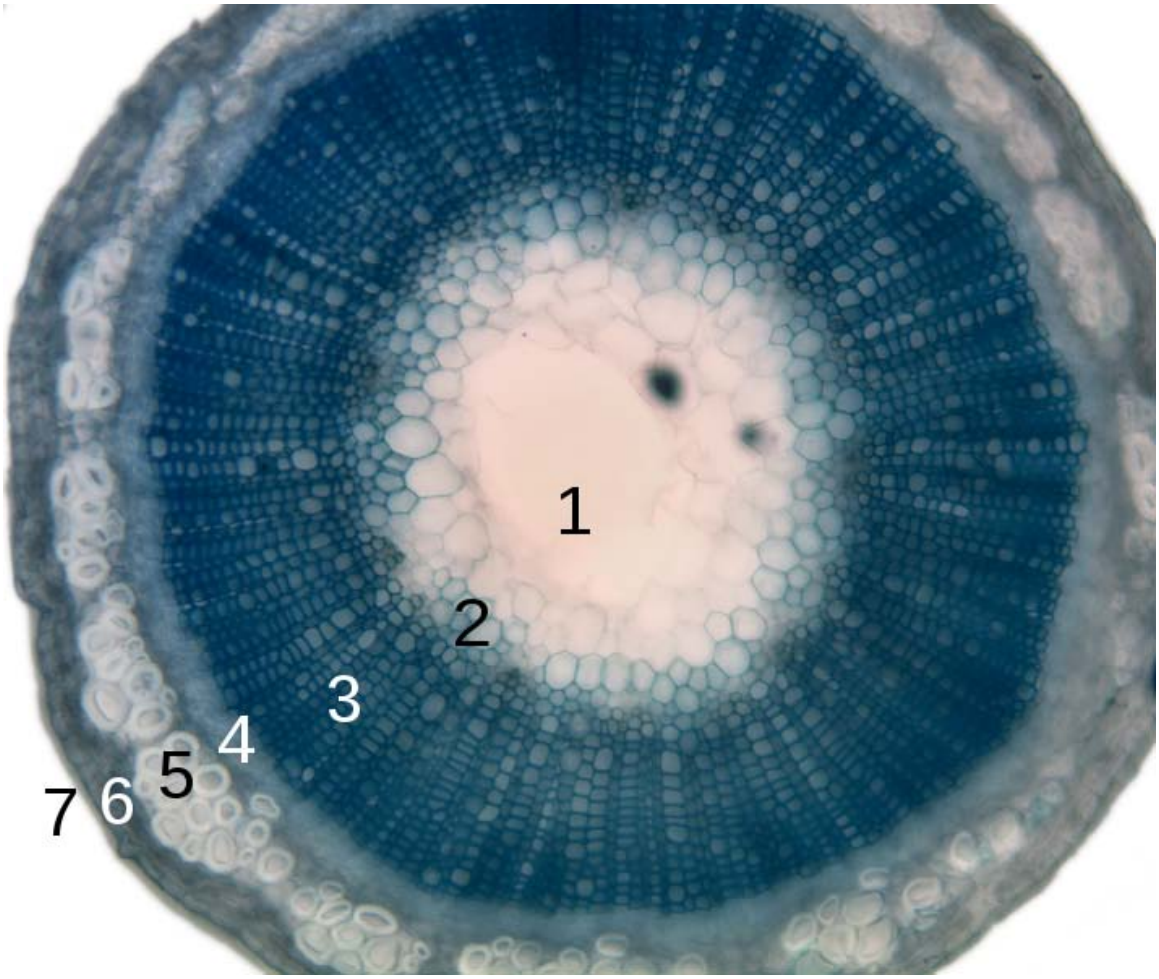
Nervous tissue

Cells comprising the central nervous system and peripheral nervous system are classified as neural tissue. In the central nervous system, neural tissue forms the brain and spinal cord and, in the peripheral nervous system forms the cranial nerves and spinal nerves, inclusive of the motor neurons. Transmits communications.

Epithelial tissue

The epithelial tissues are formed by cells that cover organ surfaces such as the surface of the skin, the airways, the reproductive tract, and the inner lining of the digestive tract. The cells comprising an epithelial layer are linked via semi-permeable, tight junctions; hence, this tissue provides a barrier between the external environment and the organ it covers. In addition to this protective function, epithelial tissue may also be specialized to function in secretion and absorption. Epithelial tissue helps to protect organisms from microorganisms, injury, and fluid loss.

Plant tissues



Cross-section of a flax plant stem with several layers of different tissue types:
1. Pith,
2. Protoxylem,
3. Xylem I,
4. Phloem I,
5. Sclerenchyma (bast fibre),
6. Cortex,
7. Epidermis

Examples of tissue in other multicellular organisms are vascular tissue in plants, such as xylem and phloem. Plant tissues are categorized broadly into three tissue systems: the epidermis, the ground tissue, and the vascular tissue. Together they are often referred to as biomass.

- **Epidermis** - Cells forming the outer surface of the leaves and of the young plant body.

- **Vascular tissue** - The primary components of vascular tissue are the xylem and phloem. These transport fluid and nutrients internally.
- **Ground tissue** - Ground tissue is less differentiated than other tissues. Ground tissue manufactures nutrients by photosynthesis and stores reserve nutrients.

Plant tissues can also be divided differently into two types:

1. Meristematic tissues
2. Permanent tissues

Meristematic tissues

Meristematic tissue consist of actively dividing cells this is found in regions such as the tips of stems or roots and lead to increase in length and thickness of the plant this cells are spherical oval polygonal and rectangular and have thin cells walls.The growth of plant occurs only in certain specific regions. At these regions, the meristematic tissues are present. New cells produced by meristem are initially those of meristem itself, but as they grow and mature, their characteristics slowly change and they become differentiated as components of other tissues. Depending on the region of occurrence of meristimatic tissues they are classified as:

a) **Apical Meristem** - It is present at the growing tips of stems and roots and increases the length of the stem and root. They form growing parts at the apices of roots and stems and are responsible for increase in length,also called primary growth.This meristem is responsible for the linear growth of an organ.
b) **Lateral Meristem** - This meristem consist of cells which mainly divide in one plane and cause the organ to increase in diameter and growth. Lateral Meristem usually occurs beneath the bark of the tree in the form of Cork Cambium and in vascular bundles of dicots in the form of vascular cambium. The activity of this cambium results in the formation of secondary growth.
c) **Intercalary Meristem** - This meristem is located in between permanent tissues. It is usually present at the base of node, inter node and on leaf base. They are responsible for growth in length of the plant.This adds growth in the girth of stem.

The cells of meristematic tissues are similar in structure and have thin and elastic primary cell wall made up of cellulose. They are compactly arranged without inter-cellular spaces between them. Each cell contains a dense cytoplasm and a prominent nucleus. Dense protoplasm of meristematic cells contains very few vacuoles. Normally the meristematic cells are oval, polygonal or rectangular in shape.

Meristemetic tissue cells have a large nucleus with small or no vacuoles, they have no inter cellular spaces.

Permanent tissues

The meristematic tissues that take up a specific role lose the ability to divide. This process of taking up a permanent shape, size and a function is called cellular differentiation. Cells of meristematic tissue differentiate to form different types of permanent tissue. There are 2 types of permanent tissues:

Simple permanent tissues

These tissues are called simple because they are composed of similar types of cells which have common origin and function. They are further classified into:

1. Parenchyma
2. Chlorenchyma
3. Aerenchyma
4. Collenchyma
5. Sclerenchyma
6. Epidermis

Parenchyma

Parenchyma is Greek word where "parn" means besides and "enchien" means to pour. Parenchyma is the most specialized primitive tissue. It mainly consist of thin-walled cells which have inter-cellular spaces between them. The cell wall is made up of cellulose. Each parenchymatous cell is iso-diametric, spherical, or oval in shape. It is widely distributed in various plant organs like root, stem, leaf, flowers and fruits. They mainly occur in the cortex epidermis, and pith, as well as in the mesophyll of leaves.

The main function of parenchymatous tissue is assimilation and storage of reserve food materials like starch, fats and proteins. They also store waste products such as gums, resins, and inorganic waste materials.

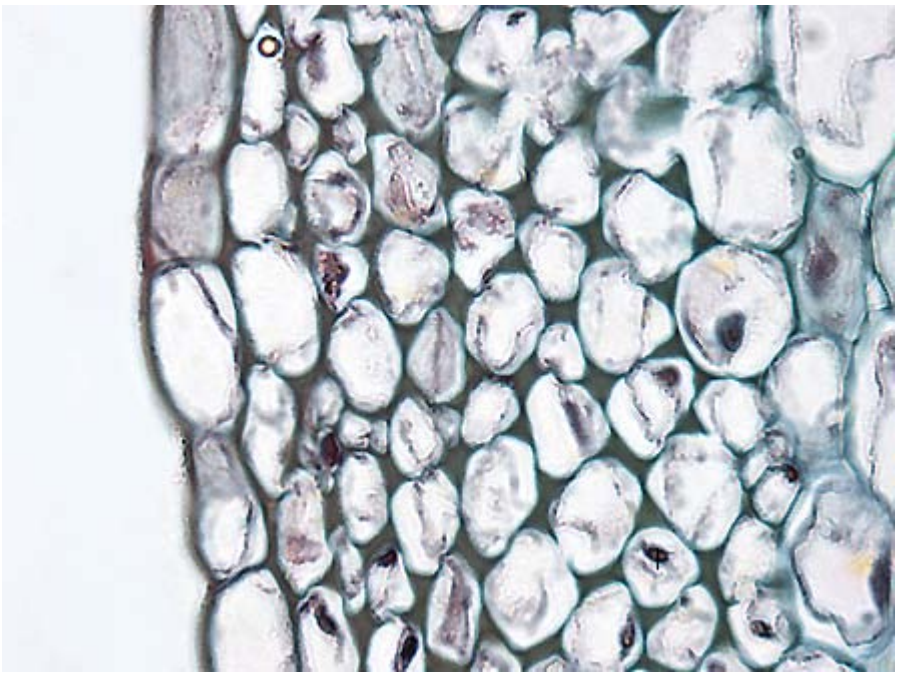
Chlorenchyma

The cells of this tissue are characterized by having chloroplasts (containing chlorophyll). It is found in the palisade and spongy tissues in the green leaves and the stem cortex of the herbs where photosynthesis occurs.

Aerenchyma

Aerenchyma is a type of parenchyma. In aquatic plants the intercellular spaces form large air cavities. They give buoyancy to the plant and help them float in water. Such parenchyma is called aerenchyma.

Collenchyma



Cross section of collenchyma cells

Collenchyma is Greek word where "Collen" means gum and "enchyma" means infusion. It is a living tissue of primary body like Parenchyma. Cells are thin-walled but possess thickening of cellulose and pectin substances at the corners where number of cells join together. This tissue gives a tensile strength to the plant and the cells are compactly arranged and do not have inter-cellular spaces. It occurs chiefly in hypodermis of stems and leaves. It is absent in monocots and in roots.

Collenchymatous tissue acts as a supporting tissue in stems of young plants. It provides mechanical support, elasticity, and tensile strength to the plant body. It helps in manufacturing sugar and storing it as starch. It is present in margin of leaves and resist tearing effect of the wind.

Sclerenchyma

Sclerenchyma is Greek word where "Sclrenes" means hard and "enchyma" means infusion. This tissue consists of thick-walled, dead cells. These cells have hard and extremely thick secondary walls due to uniform distribution of lignin. Lignin deposition is so thick that the cell walls become strong, rigid and impermeable to water. Sclerenchymatous cells are closely packed without inter-cellular spaces between them. Thus, they appear as hexagonal net in transverse section. The cells are cemented with the help of lamella. The middle lamella is a wall that lies between adjacent cells. Sclerenchymatous cells mainly occur in hypodermis, pericycle, secondary xylem and phloem. They also occur in endocorp of almond and coconut. It is made of pectin, lignin, protein. The cells of sclerenchymatous cells can be classified as :

1. Fibres- Fibres are long, elongated sclerenchymatous cells with pointed ends.
2. Sclerides- Sclerenchymatous cells which are short and possess extremely thick, lamellated, lignified walls with long singular piths. They are called sclerides.

The main function of Sclerenchymatous tissues is to give support to the plant.

Epidermis

The entire surface of the plant consists of a single layer of cells called epidermis or surface tissue. The entire surface of the plant has this outer layer of epidermis. Hence it is also called surface tissue. Most of the epidermal cells are relatively flat. the outer and lateral walls of the cell are often thicker than the inner walls. The cells forms a continuous sheet without inter cellular spaces. It protects all parts of the plant.

Complex permanent tissue

A complex permanent tissue may be classified as a group of more than one type of tissue having a common origin and working together as a unit to perform a function. These tissues are concerned with transportation of water, mineral, nutrients and organic substances. The important complex tissues in vascular plants are xylem, phloem.

Xylem

Xylem is a chief, conducting tissue of vascular plants. It is responsible for conduction of water and inorganic solutes.

Xylem is an important plant tissue as it is part of the 'plumbing' of a plant. Think of bundles of pipes running along the main axis of stems and roots. It carries water and dissolved substances throughout and consists of a combination of parenchyma cells, fibers, vessels, tracheids and ray cells. Long tubes made up of individual cells are the vessels, while vessel members are open at each end. Internally, there may be bars of wall material extending across the open space. These cells are joined end to end to form long tubes. Vessel members and tracheids are dead at maturity. Tracheids have thick secondary cell walls and are tapered at the ends. They do not have end openings such as the vessels. The tracheids ends overlap with each other, with pairs of pits present. The pit pairs allow water to pass from cell to cell. While most conduction in the xylem is up and down, there is some side-to-side or lateral conduction via rays. Rays are horizontal rows of long-living parenchyma cells that arise out of the vascular cambium. In trees, and other woody plants, ray will radiate out from the center of stems and roots and in cross-section will look like the spokes of a wheel.

Phloem

Phloem is an equally important plant tissue as it also is part of the 'plumbing' of a plant. Primarily, phloem carries dissolved food substances throughout the plant. This conduction system is composed of sieve-tube member and companion cells, that are

without secondary walls. The parent cells of the vascular cambium produce both xylem and phloem. This usually also includes fibers, parenchyma and ray cells. Sieve tubes are formed from sieve-tube members laid end to end. The end walls, unlike vessel members in xylem, do not have openings. The end walls, however, are full of small pores where cytoplasm extends from cell to cell. These porous connections are called sieve plates. In spite of the fact that their cytoplasm is actively involved in the conduction of food materials, sieve-tube members do not have nuclei at maturity. It is the companion cells that are nestled between sieve-tube members that function in some manner bringing about the conduction of food. Sieve-tube members that are alive contain a polymer called callose. Callose stays in solution as long at the cell contents are under pressure. As a repair mechanism, if an insect injures a cell and the pressure drops, the callose will precipitate. However, the callose and a phloem protein will be moved through the nearest sieve plate where they will form a plug. This prevents further leakage of sieve tube contents and the injury is not necessarily fatal to overall plant turgor pressure. Phloem transports food and materials in plants in upwards and downwards as required.

Chapter- 3

Cellular Differentiation

In developmental biology, **cellular differentiation** is the process by which a less specialized cell becomes a more specialized cell type. Differentiation occurs numerous times during the development of a multicellular organism as the organism changes from a simple zygote to a complex system of tissues and cell types. Differentiation is a common process in adults as well: adult stem cells divide and create fully-differentiated daughter cells during tissue repair and during normal cell turnover. Differentiation dramatically changes a cell's size, shape, membrane potential, metabolic activity, and responsiveness to signals. These changes are largely due to highly-controlled modifications in gene expression. With a few exceptions, cellular differentiation almost never involves a change in the DNA sequence itself. Thus, different cells can have very different physical characteristics despite having the same genome.

A cell that is able to differentiate into all cell types of the adult organism is known as *pluripotent*. Such cells are called embryonic stem cells in animals and meristematic cells in higher plants. A cell that is able to differentiate into all cell types, including the placental tissue, is known as *totipotent*. In mammals, only the zygote and subsequent blastomeres are totipotent, while in plants many differentiated cells can become totipotent with simple laboratory techniques. In cytopathology, the level of cellular differentiation is used as a measure of cancer progression. "Grade" is a marker of how differentiated a cell in a tumor is.

Mammalian cell types

Three basic categories of cells make up the mammalian body: germ cells, somatic cells, and stem cells. Each of the approximately 100 trillion (10^{14}) cells in an adult human has its own copy or copies of the genome except certain cell types, such as red blood cells, that lack nuclei in their fully differentiated state. Most cells are diploid; they have two copies of each chromosome. Such cells, called somatic cells, make up most of the human body, such as skin and muscle cells. Cells differentiate to specialize for different functions.

Germ line cells are any line of cells that give rise to gametes—eggs and sperm—and thus are continuous through the generations. Stem cells, on the other hand, have the ability to divide for indefinite periods and to give rise to specialized cells. They are best described in the context of normal human development.

Development begins when a sperm fertilizes an egg and creates a single cell that has the potential to form an entire organism. In the first hours after fertilization, this cell divides into identical cells. In humans, approximately four days after fertilization and after several cycles of cell division, these cells begin to specialize, forming a hollow sphere of cells, called a blastocyst. The blastocyst has an outer layer of cells, and inside this hollow sphere, there is a cluster of cells called the inner cell mass. The cells of the inner cell mass go on to form virtually all of the tissues of the human body. Although the cells of the inner cell mass can form virtually every type of cell found in the human body, they cannot form an organism. These cells are referred to as pluripotent.

Pluripotent stem cells undergo further specialization into multipotent progenitor cells that then give rise to functional cells. Examples of stem and progenitor cells include:

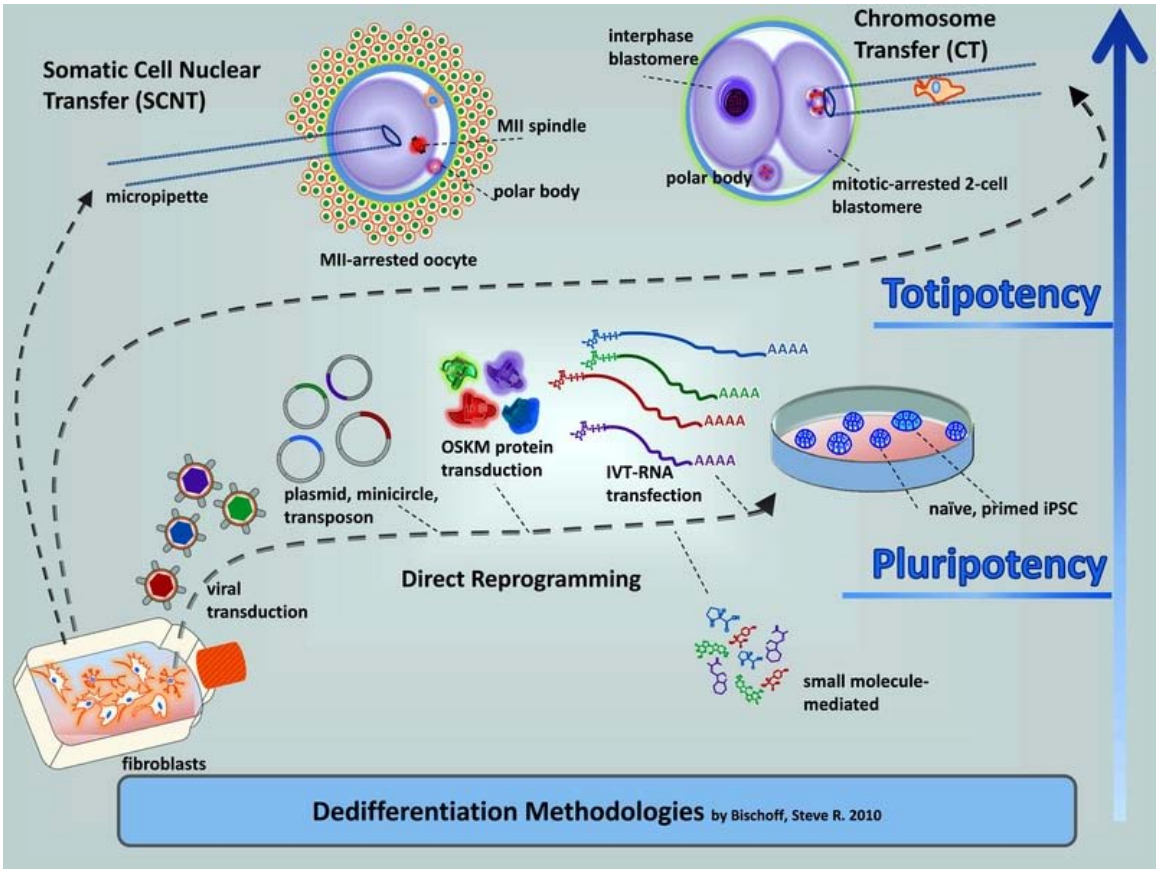
- *Hematopoietic stem cells* (adult stem cells) from the bone marrow that give rise to red blood cells, white blood cells, and platelets
- *Mesenchymal stem cells* (adult stem cells) from the bone marrow that give rise to stromal cells, fat cells, and types of bone cells
- *Epithelial stem cells* (progenitor cells) that give rise to the various types of skin cells
- *Muscle satellite cells* (progenitor cells) that contribute to differentiated muscle tissue

Dedifferentiation

Dedifferentiation is a cellular process often seen in more basal life forms such as worms and amphibians in which a partially or terminally differentiated cell reverts to an earlier developmental stage, usually as part of a regenerative process. Dedifferentiation also occurs in plants. Cells in cell culture can lose properties they originally had, such as protein expression, or change shape. This process is also termed dedifferentiation.

Some believe dedifferentiation is an aberration of the normal development cycle that results in cancer, whereas others believe it to be a natural part of the immune response lost by humans at some point as a result of evolution.

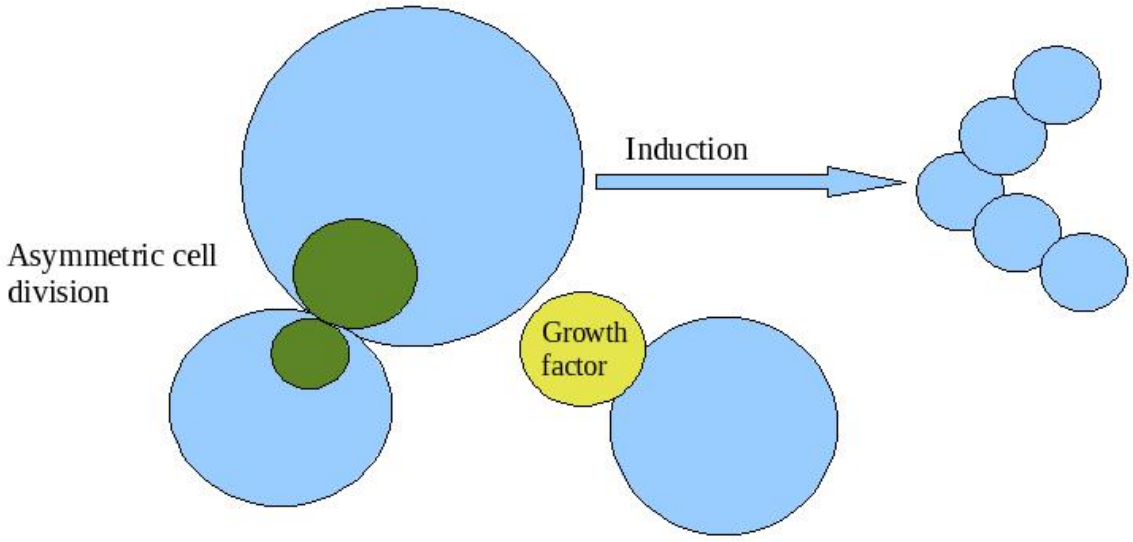
A small molecule dubbed reversine, a purine analog, has been discovered that has proven to induce dedifferentiation in myotubes. These dedifferentiated cells were then able to redifferentiate into osteoblasts and adipocytes.



Dedifferentiation to totipotency or pluripotency: an overview of methods. Various methods exist to revert adult somatic cells to pluripotency or totipotency. In the case of totipotency, reprogramming is mediated through a mature metaphase II oocyte as in somatic cell nuclear transfer (Wilmut et al., 1997). Recent work has demonstrated the feasibility of enucleated zygotes or early blastomeres chemically arrested during mitosis, such that nuclear envelope break down occurs, to support reprogramming to totipotency in a process called chromosome transfer (Egli and Eggan, 2010). Direct reprogramming methods support reversion to pluripotency; though, vehicles and biotypes vary considerably in efficiencies (Takahashi and Yamanaka, 2006). Viral-mediated transduction robustly supports dedifferentiation to pluripotency through retroviral or DNA-viral routes but carries the onus of insertional inactivation. Additionally, epigenetic reprogramming by enforced expression of OSKM through DNA routes exists such as plasmid DNA, minicircles, transposons, episomes and DNA mulicistronic construct targeting by homologous recombination has also been demonstrated; however, these methods suffer from the burden to potentially alter the recipient genome by gene insertion (Ho et al., 2010). While protein-mediated transduction supports reprogramming adult cells to pluripotency, the method is cumbersome and requires recombinant protein expression and purification expertise, and reprograms albeit at very low frequencies (Kim et al., 2009). A major obstacle of using RNA for reprogramming is its lability and that single-stranded RNA biotypes trigger innate antiviral defense pathways such as interferon and NF-kB-dependent pathways. In vitro transcribed RNA, containing stabilizing modifications such as 5-methylguanosine capping or substituted ribonucleobases, e.g.

pseudouracil, is 35-fold more efficient than viral transduction and has the additional benefit of not altering the somatic genome (Warren et al., 2010).

Mechanisms



Mechanisms of cellular differentiation

Each specialized cell type in an organism expresses a subset of all the genes that constitute the genome of that species. Each cell type is defined by its particular pattern of regulated gene expression. Cell differentiation is thus a transition of a cell from one cell type to another and it involves a switch from one pattern of gene expression to another. Cellular differentiation during development can be understood as the result of a gene regulatory network. A regulatory gene and its cis-regulatory modules are nodes in a gene regulatory network; they receive input and create output elsewhere in the network. The systems biology approach to developmental biology emphasizes the importance of investigating how developmental mechanisms interact to produce predictable patterns (morphogenesis). (However, an alternative view has been proposed recently. Based on stochastic gene expression, cellular differentiation is the result of a Darwinian selective process occurring among cells. In this frame, protein and gene networks are the result of cellular processes and not their cause. See: Cellular Darwinism)

A few evolutionarily conserved types of molecular processes are often involved in the cellular mechanisms that control these switches. The major types of molecular processes that control cellular differentiation involve cell signaling. Many of the signal molecules that convey information from cell to cell during the control of cellular differentiation are called growth factors. Although the details of specific signal transduction pathways vary, these pathways often share the following general steps. A ligand produced by one cell binds to a receptor in the extracellular region of another cell, inducing a conformational

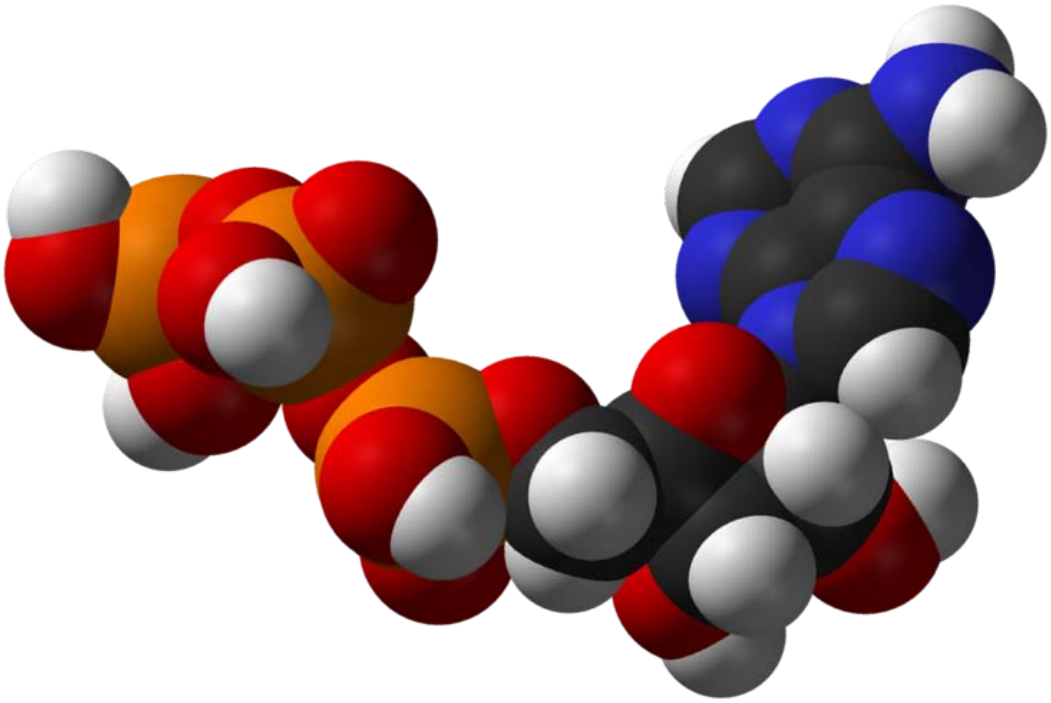
change in the receptor. The shape of the cytoplasmic domain of the receptor changes, and the receptor acquires enzymatic activity. The receptor then catalyzes reactions that phosphorylate other proteins, activating them. A cascade of phosphorylation reactions eventually activates a dormant transcription factor or cytoskeletal protein, thus contributing to the differentiation process in the target cell. Cells and tissues can vary in competence, their ability to respond to external signals.

Induction refers to cascades of signaling events, during which a cell or tissue signals to another cell or tissue to influence its developmental fate. Yamamoto and Jeffery investigated the role of the lens in eye formation in cave- and surface-dwelling fish, a striking example of induction. Through reciprocal transplants, Yamamoto and Jeffery found that the lens vesicle of surface fish can induce other parts of the eye to develop in cave- and surface-dwelling fish, while the lens vesicle of the cave-dwelling fish cannot.

Other important mechanisms fall under the category of asymmetric cell divisions, divisions that give rise to daughter cells with distinct developmental fates. Asymmetric cell divisions can occur because of segregation of cytoplasmic determinants or because of signaling. In the former mechanism, distinct daughter cells are created during cytokinesis because of an uneven distribution of regulatory molecules in the parent cell; the distinct cytoplasm that each daughter cell inherits results in a distinct pattern of differentiation for each daughter cell. A well-studied example of pattern formation by asymmetric divisions is body axis patterning in Drosophila. RNA molecules are an important type of intracellular differentiation control signal. The molecular and genetic basis of asymmetric cell divisions has also been studied in green algae of the genus *Volvox*, a model system for studying how unicellular organisms can evolve into multicellular organisms. In *Volvox carteri*, the 16 cells in the anterior hemisphere of a 32-celled embryo divide asymmetrically, each producing one large and one small daughter cell. The size of the cell at the end of all cell divisions determines whether it becomes a specialized germ or somatic cell.

Chapter- 4

Metabolism



Structure of the coenzyme adenosine triphosphate, a central intermediate in energy metabolism

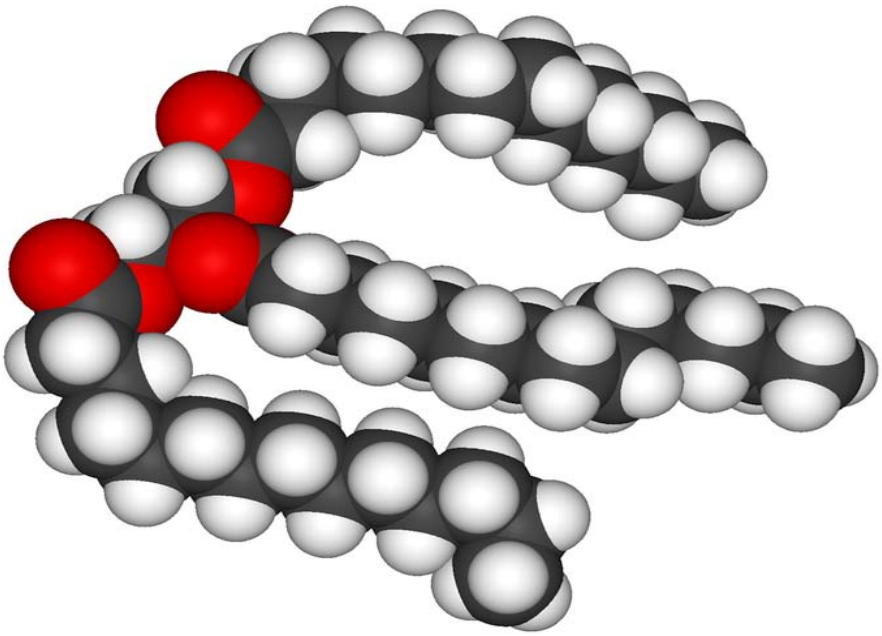
Metabolism is the set of chemical reactions that happen in living organisms to maintain life. These processes allow organisms to grow and reproduce, maintain their structures, and respond to their environments. Metabolism is usually divided into two categories. Catabolism breaks down organic matter, for example to harvest energy in cellular respiration. Anabolism uses energy to construct components of cells such as proteins and nucleic acids.

The chemical reactions of metabolism are organized into metabolic pathways, in which one chemical is transformed through a series of steps into another chemical, by a sequence of enzymes. Enzymes are crucial to metabolism because they allow organisms to drive desirable reactions that require energy and will not occur by themselves, by coupling them to spontaneous reactions that release energy. As enzymes act as catalysts they allow these reactions to proceed quickly and efficiently. Enzymes also allow the regulation of metabolic pathways in response to changes in the cell's environment or signals from other cells.

The metabolism of an organism determines which substances it will find nutritious and which it will find poisonous. For example, some prokaryotes use hydrogen sulfide as a nutrient, yet this gas is poisonous to animals. The speed of metabolism, the metabolic rate, also influences how much food an organism will require.

A striking feature of metabolism is the similarity of the basic metabolic pathways and components between even vastly different species. For example, the set of carboxylic acids that are best known as the intermediates in the citric acid cycle are present in all organisms, being found in species as diverse as the unicellular bacteria *Escherichia coli* and huge multicellular organisms like elephants. These striking similarities in metabolism are probably due to the high efficiency of these pathways, and their early appearance in evolutionary history.

Key biochemicals



Structure of a triacylglycerol lipid

Most of the structures that make up animals, plants and microbes are made from three basic classes of molecule: amino acids, carbohydrates and lipids (often called fats). As these molecules are vital for life, metabolic reactions focus on making these molecules during the construction of cells and tissues, or breaking them down and using them as a source of energy, in the digestion and use of food. Many important biochemicals can be joined together to make polymers such as DNA and proteins. These macromolecules are essential.

Type of molecule	Name of monomer forms	Name of polymer forms	Examples of polymer forms
Amino acids	Amino acids	Proteins (also called polypeptides)	Fibrous proteins and globular proteins
Carbohydrates	Monosaccharides	Polysaccharides	Starch, glycogen and cellulose
Nucleic acids	Nucleotides	Polynucleotides	DNA and RNA

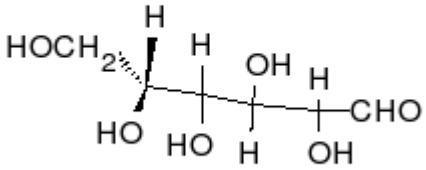
Amino acids and proteins

Proteins are made of amino acids arranged in a linear chain and joined together by peptide bonds. Many proteins are the enzymes that catalyze the chemical reactions in metabolism. Other proteins have structural or mechanical functions, such as the proteins that form the cytoskeleton, a system of scaffolding that maintains the cell shape. Proteins are also important in cell signaling, immune responses, cell adhesion, active transport across membranes, and the cell cycle.

Lipids

Lipids are the most diverse group of biochemicals. Their main structural uses are as part of biological membranes such as the cell membrane, or as a source of energy. Lipids are usually defined as hydrophobic or amphipathic biological molecules that will dissolve in organic solvents such as benzene or chloroform. The fats are a large group of compounds that contain fatty acids and glycerol; a glycerol molecule attached to three fatty acid esters is a triacylglyceride. Several variations on this basic structure exist, including alternate backbones such as sphingosine in the sphingolipids, and hydrophilic groups such as phosphate in phospholipids. Steroids such as cholesterol are another major class of lipids that are made in cells.

Carbohydrates



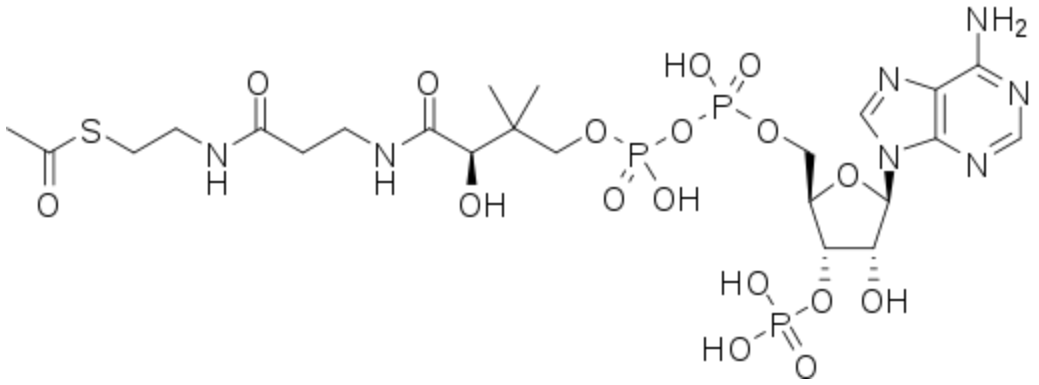
Glucose can exist in both a straight-chain and ring form.

Carbohydrates are straight-chain aldehydes or ketones with many hydroxyl groups that can exist as straight chains or rings. Carbohydrates are the most abundant biological molecules, and fill numerous roles, such as the storage and transport of energy (starch, glycogen) and structural components (cellulose in plants, chitin in animals). The basic carbohydrate units are called monosaccharides and include galactose, fructose, and most importantly glucose. Monosaccharides can be linked together to form polysaccharides in almost limitless ways.

Nucleotides

The two nucleic acids, DNA and RNA are polymers of nucleotides, each nucleotide comprising a phosphate group, a ribose sugar group, and a nitrogenous base. Nucleic acids are critical for the storage and use of genetic information, through the processes of transcription and protein biosynthesis. This information is protected by DNA repair mechanisms and propagated through DNA replication. Many viruses have an RNA genome, for example HIV, which uses reverse transcription to create a DNA template from its viral RNA genome. RNA in ribozymes such as spliceosomes and ribosomes is similar to enzymes as it can catalyze chemical reactions. Individual nucleosides are made by attaching a nucleobase to a ribose sugar. These bases are heterocyclic rings containing nitrogen, classified as purines or pyrimidines. Nucleotides also act as coenzymes in metabolic group transfer reactions.

Coenzymes

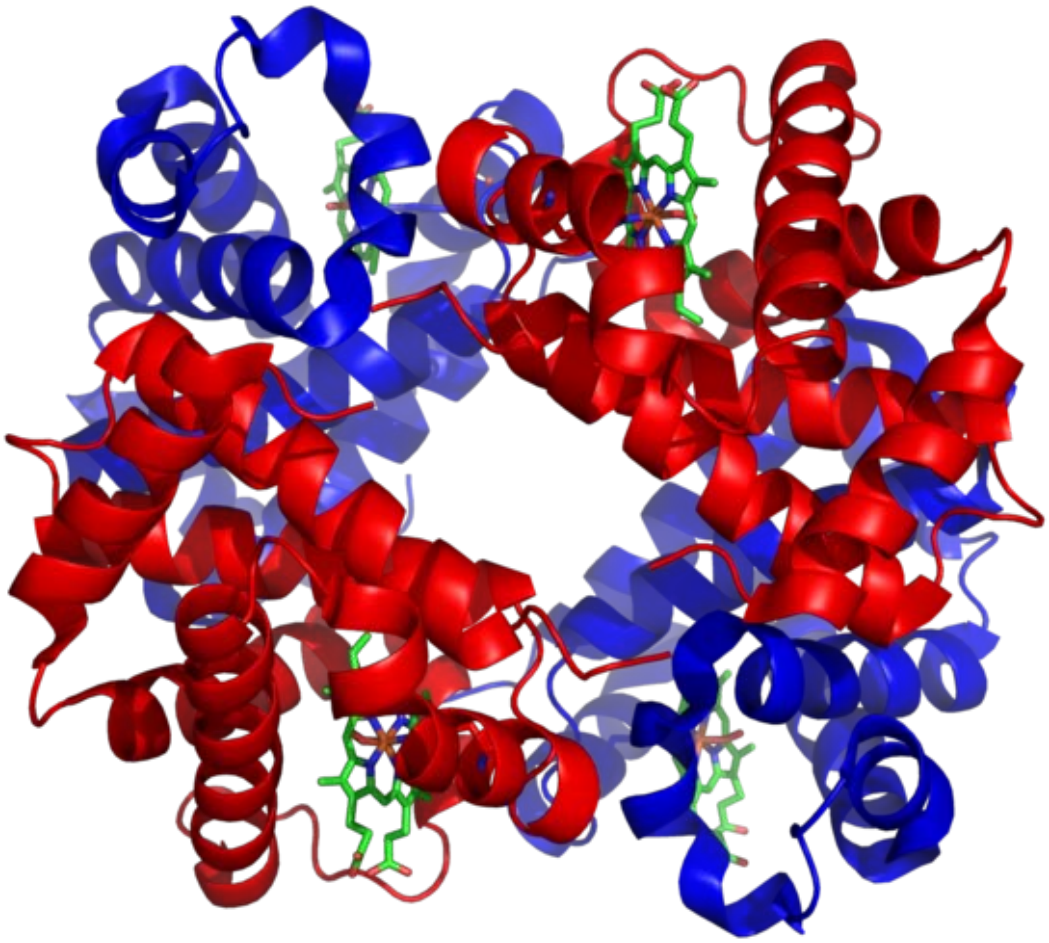


Structure of the coenzyme acetyl-CoA.The transferable acetyl group is bonded to the sulfur atom at the extreme left.

Metabolism involves a vast array of chemical reactions, but most fall under a few basic types of reactions that involve the transfer of functional groups. This common chemistry allows cells to use a small set of metabolic intermediates to carry chemical groups between different reactions. These group-transfer intermediates are called coenzymes. Each class of group-transfer reaction is carried out by a particular coenzyme, which is the substrate for a set of enzymes that produce it, and a set of enzymes that consume it. These coenzymes are therefore continuously being made, consumed and then recycled.

One central coenzyme is adenosine triphosphate (ATP), the universal energy currency of cells. This nucleotide is used to transfer chemical energy between different chemical reactions. There is only a small amount of ATP in cells, but as it is continuously regenerated, the human body can use about its own weight in ATP per day. ATP acts as a bridge between catabolism and anabolism, with catabolic reactions generating ATP and anabolic reactions consuming it. It also serves as a carrier of phosphate groups in phosphorylation reactions.

A vitamin is an organic compound needed in small quantities that cannot be made in the cells. In human nutrition, most vitamins function as coenzymes after modification; for example, all water-soluble vitamins are phosphorylated or are coupled to nucleotides when they are used in cells. Nicotinamide adenine dinucleotide (NADH), a derivative of vitamin B_3 (niacin), is an important coenzyme that acts as a hydrogen acceptor. Hundreds of separate types of dehydrogenases remove electrons from their substrates and reduce NAD^+ into NADH. This reduced form of the coenzyme is then a substrate for any of the reductases in the cell that need to reduce their substrates. Nicotinamide adenine dinucleotide exists in two related forms in the cell, NADH and NADPH. The NAD^+/NADH form is more important in catabolic reactions, while $NADP^+$/NADPH is used in anabolic reactions.



Structure of hemoglobin. The protein subunits are in red and blue, and the iron-containing heme groups in green. From PDB 1GZX.

Minerals and cofactors

Inorganic elements play critical roles in metabolism; some are abundant (e.g. sodium and potassium) while others function at minute concentrations. About 99% of mammals' mass are the elements carbon, nitrogen, calcium, sodium, chlorine, potassium, hydrogen, phosphorus, oxygen and sulfur. The organic compounds (proteins, lipids and carbohydrates) contain the majority of the carbon and nitrogen and most of the oxygen and hydrogen is present as water.

The abundant inorganic elements act as ionic electrolytes. The most important ions are sodium, potassium, calcium, magnesium, chloride, phosphate, and the organic ion

bicarbonate. The maintenance of precise gradients across cell membranes maintains osmotic pressure and pH. Ions are also critical for nerves and muscles, as action potentials in these tissues are produced by the exchange of electrolytes between the extracellular fluid and the cytosol. Electrolytes enter and leave cells through proteins in the cell membrane called ion channels. For example, muscle contraction depends upon the movement of calcium, sodium and potassium through ion channels in the cell membrane and T-tubules.

The transition metals are usually present as trace elements in organisms, with zinc and iron being most abundant. These metals are used in some proteins as cofactors and are essential for the activity of enzymes such as catalase and oxygen-carrier proteins such as hemoglobin. These cofactors are bound tightly to a specific protein; although enzyme cofactors can be modified during catalysis, cofactors always return to their original state after catalysis has taken place. The metal micronutrients are taken up into organisms by specific transporters and bound to storage proteins such as ferritin or metallothionein when not being used.

Catabolism

Catabolism is the set of metabolic processes that break down large molecules. These include breaking down and oxidizing food molecules. The purpose of the catabolic reactions is to provide the energy and components needed by anabolic reactions. The exact nature of these catabolic reactions differ from organism to organism and organisms can be classified based on their sources of energy and carbon (their primary nutritional groups), as shown in the table below. Organic molecules being used as a source of energy in organotrophs, while lithotrophs use inorganic substrates and phototrophs capture sunlight as chemical energy. However, all these different forms of metabolism depend on redox reactions that involve the transfer of electrons from reduced donor molecules such as organic molecules, water, ammonia, hydrogen sulfide or ferrous ions to acceptor molecules such as oxygen, nitrate or sulfate. In animals these reactions involve complex organic molecules being broken down to simpler molecules, such as carbon dioxide and water. In photosynthetic organisms such as plants and cyanobacteria, these electron-transfer reactions do not release energy, but are used as a way of storing energy absorbed from sunlight.

Classification of organisms based on their metabolism

energy source	**sunlight**	**photo-**			**-troph**
	preformed molecules	**chemo-**			
electron donor	**organic compound**		**organo-**		
	inorganic compound		**litho-**		
carbon source	**organic compound**			**hetero-**	

inorganic compound

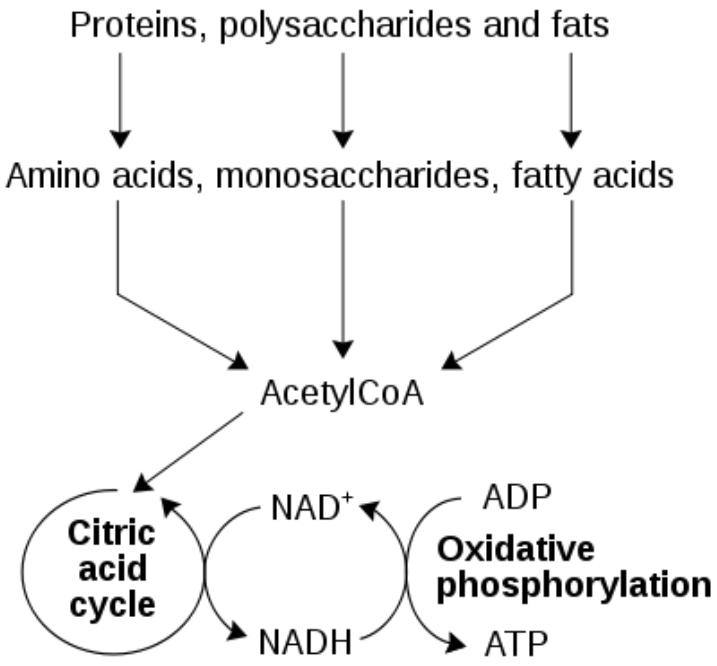
auto-

The most common set of catabolic reactions in animals can be separated into three main stages. In the first, large organic molecules such as proteins, polysaccharides or lipids are digested into their smaller components outside cells. Next, these smaller molecules are taken up by cells and converted to yet smaller molecules, usually acetyl coenzyme A (acetyl-CoA), which releases some energy. Finally, the acetyl group on the CoA is oxidised to water and carbon dioxide in the citric acid cycle and electron transport chain, releasing the energy that is stored by reducing the coenzyme nicotinamide adenine dinucleotide (NAD^+) into NADH.

Digestion

Macromolecules such as starch, cellulose or proteins cannot be rapidly taken up by cells and need to be broken into their smaller units before they can be used in cell metabolism. Several common classes of enzymes digest these polymers. These digestive enzymes include proteases that digest proteins into amino acids, as well as glycoside hydrolases that digest polysaccharides into monosaccharides.

Microbes simply secrete digestive enzymes into their surroundings, while animals only secrete these enzymes from specialized cells in their guts. The amino acids or sugars released by these extracellular enzymes are then pumped into cells by specific active transport proteins.



A simplified outline of the catabolism of proteins, carbohydrates and fats

Energy from organic compounds

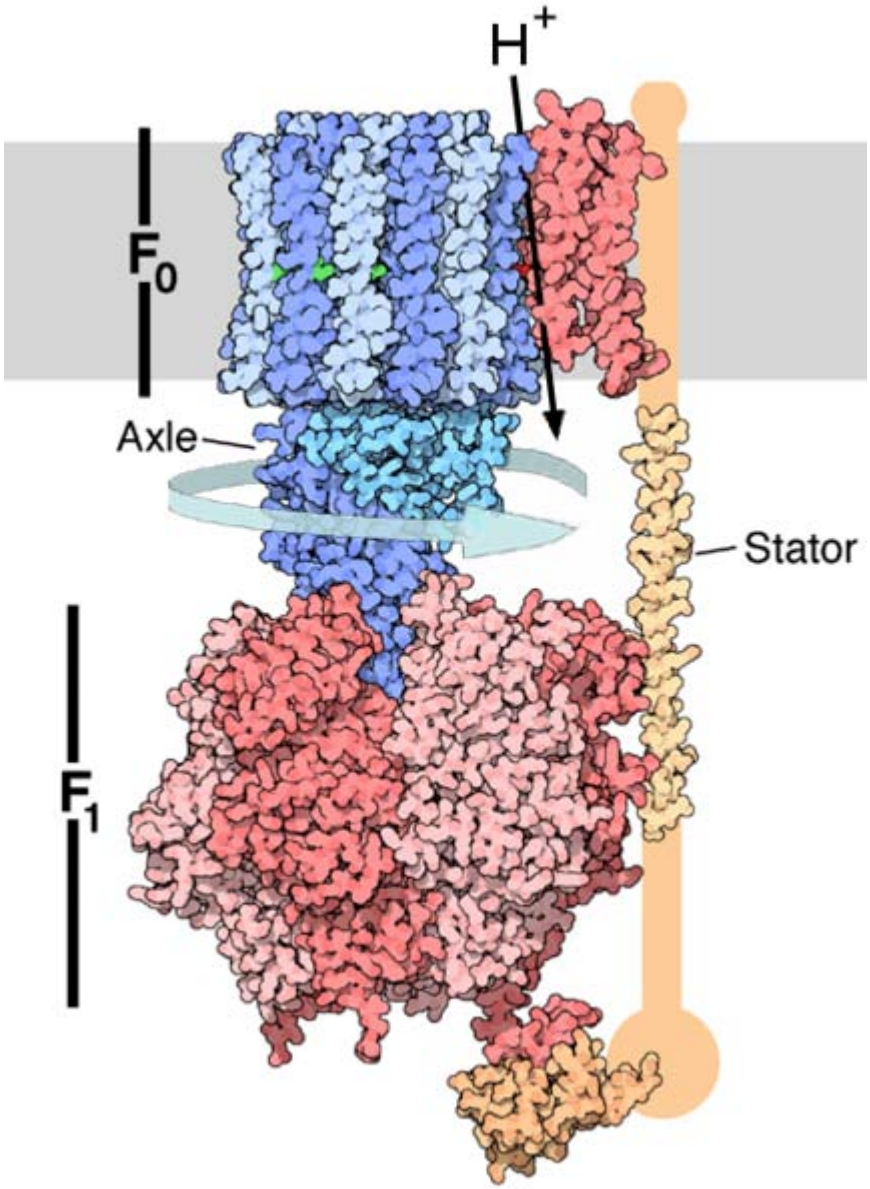
Carbohydrate catabolism is the breakdown of carbohydrates into smaller units. Carbohydrates are usually taken into cells once they have been digested into monosaccharides. Once inside, the major route of breakdown is glycolysis, where sugars such as glucose and fructose are converted into pyruvate and some ATP is generated. Pyruvate is an intermediate in several metabolic pathways, but the majority is converted to acetyl-CoA and fed into the citric acid cycle. Although some more ATP is generated in the citric acid cycle, the most important product is NADH, which is made from NAD^+ as the acetyl-CoA is oxidized. This oxidation releases carbon dioxide as a waste product. In anaerobic conditions, glycolysis produces lactate, through the enzyme lactate dehydrogenase re-oxidizing NADH to NAD+ for re-use in glycolysis. An alternative route for glucose breakdown is the pentose phosphate pathway, which reduces the coenzyme NADPH and produces pentose sugars such as ribose, the sugar component of nucleic acids.

Fats are catabolised by hydrolysis to free fatty acids and glycerol. The glycerol enters glycolysis and the fatty acids are broken down by beta oxidation to release acetyl-CoA, which then is fed into the citric acid cycle. Fatty acids release more energy upon oxidation than carbohydrates because carbohydrates contain more oxygen in their structures.

Amino acids are either used to synthesize proteins and other biomolecules, or oxidized to urea and carbon dioxide as a source of energy. The oxidation pathway starts with the removal of the amino group by a transaminase. The amino group is fed into the urea cycle, leaving a deaminated carbon skeleton in the form of a keto acid. Several of these keto acids are intermediates in the citric acid cycle, for example the deamination of glutamate forms α-ketoglutarate. The glucogenic amino acids can also be converted into glucose, through gluconeogenesis.

Energy transformations

Oxidative phosphorylation



Structure of ATP synthase. The proton channel and rotating stalk are shown in blue and the synthase subunits in red.

In oxidative phosphorylation, the electrons removed from food molecules in pathways such as the citric acid cycle are transferred to oxygen and the energy released is used to make ATP. This is done in eukaryotes by a series of proteins in the membranes of mitochondria called the electron transport chain. In prokaryotes, these proteins are found in the cell's inner membrane. These proteins use the energy released from passing

electrons from reduced molecules like NADH onto oxygen to pump protons across a membrane.

Pumping protons out of the mitochondria creates a proton concentration difference across the membrane and generates an electrochemical gradient. This force drives protons back into the mitochondrion through the base of an enzyme called ATP synthase. The flow of protons makes the stalk subunit rotate, causing the active site of the synthase domain to change shape and phosphorylate adenosine diphosphate - turning it into ATP.

Energy from inorganic compounds

Chemolithotrophy is a type of metabolism found in prokaryotes where energy is obtained from the oxidation of inorganic compounds. These organisms can use hydrogen, reduced sulfur compounds (such as sulfide, hydrogen sulfide and thiosulfate), ferrous iron (FeII) or ammonia as sources of reducing power and they gain energy from the oxidation of these compounds with electron acceptors such as oxygen or nitrite. These microbial processes are important in global biogeochemical cycles such as acetogenesis, nitrification and denitrification and are critical for soil fertility.

Energy from light

The energy in sunlight is captured by plants, cyanobacteria, purple bacteria, green sulfur bacteria and some protists. This process is often coupled to the conversion of carbon dioxide into organic compounds, as part of photosynthesis, which is discussed below. The energy capture and carbon fixation systems can however operate separately in prokaryotes, as purple bacteria and green sulfur bacteria can use sunlight as a source of energy, while switching between carbon fixation and the fermentation of organic compounds.

In many organisms the capture of solar energy is similar in principle to oxidative phosphorylation, as it involves energy being stored as a proton concentration gradient and this proton motive force then driving ATP synthesis. The electrons needed to drive this electron transport chain come from light-gathering proteins called photosynthetic reaction centres or rhodopsins. Reaction centers are classed into two types depending on the type of photosynthetic pigment present, with most photosynthetic bacteria only having one type, while plants and cyanobacteria have two.

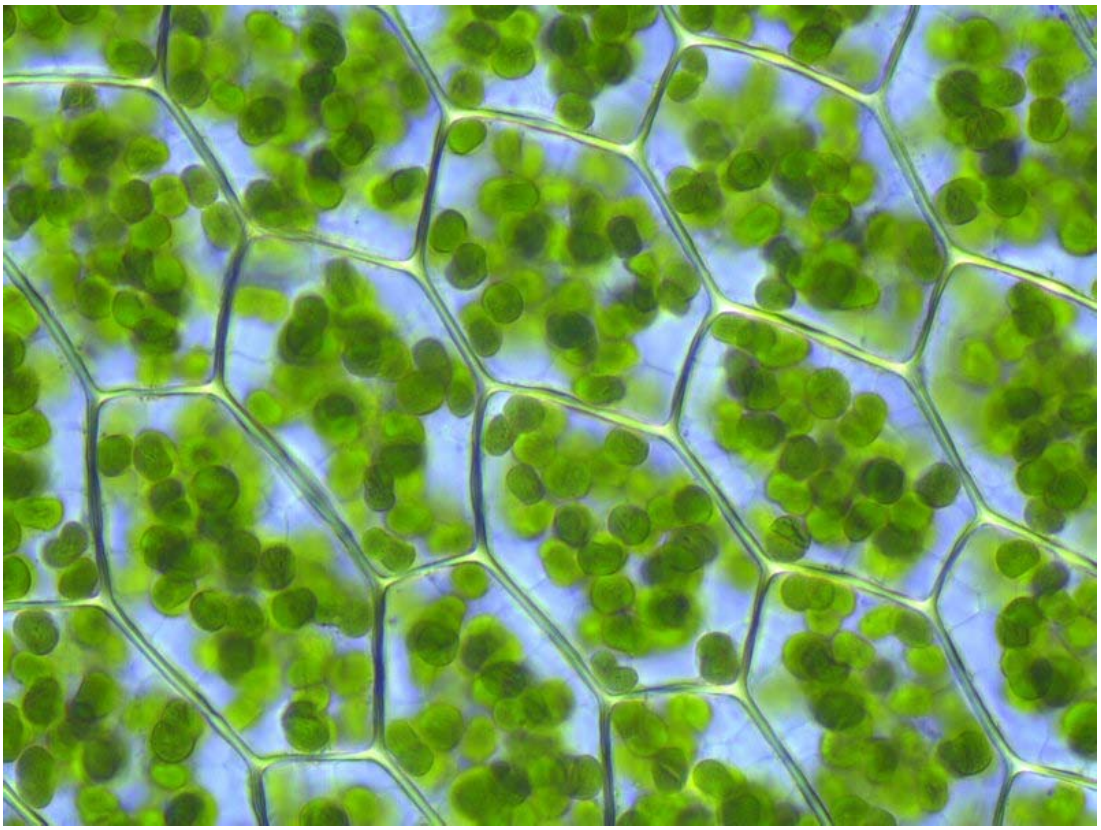
In plants, algae, and cyanobacteria, photosystem II uses light energy to remove electrons from water, releasing oxygen as a waste product. The electrons then flow to the cytochrome b6f complex, which uses their energy to pump protons across the thylakoid membrane in the chloroplast. These protons move back through the membrane as they drive the ATP synthase, as before. The electrons then flow through photosystem I and can then either be used to reduce the coenzyme $NADP^+$, for use in the Calvin cycle which is discussed below, or recycled for further ATP generation.

Anabolism

Anabolism is the set of constructive metabolic processes where the energy released by catabolism is used to synthesize complex molecules. In general, the complex molecules that make up cellular structures are constructed step-by-step from small and simple precursors. Anabolism involves three basic stages. Firstly, the production of precursors such as amino acids, monosaccharides, isoprenoids and nucleotides, secondly, their activation into reactive forms using energy from ATP, and thirdly, the assembly of these precursors into complex molecules such as proteins, polysaccharides, lipids and nucleic acids.

Organisms differ in how many of the molecules in their cells they can construct for themselves. Autotrophs such as plants can construct the complex organic molecules in cells such as polysaccharides and proteins from simple molecules like carbon dioxide and water. Heterotrophs, on the other hand, require a source of more complex substances, such as monosaccharides and amino acids, to produce these complex molecules. Organisms can be further classified by ultimate source of their energy: photoautotrophs and photoheterotrophs obtain energy from light, whereas chemoautotrophs and chemoheterotrophs obtain energy from inorganic oxidation reactions.

Carbon fixation



Plant cells (bounded by purple walls) filled with chloroplasts (green), which are the site of photosynthesis

Photosynthesis is the synthesis of carbohydrates from sunlight and carbon dioxide (CO_2). In plants, cyanobacteria and algae, oxygenic photosynthesis splits water, with oxygen produced as a waste product. This process uses the ATP and NADPH produced by the photosynthetic reaction centres, as described above, to convert CO_2 into glycerate 3-phosphate, which can then be converted into glucose. This carbon-fixation reaction is carried out by the enzyme RuBisCO as part of the Calvin – Benson cycle. Three types of photosynthesis occur in plants, C3 carbon fixation, C4 carbon fixation and CAM photosynthesis. These differ by the route that carbon dioxide takes to the Calvin cycle, with C3 plants fixing CO_2 directly, while C4 and CAM photosynthesis incorporate the CO_2 into other compounds first, as adaptations to deal with intense sunlight and dry conditions.

In photosynthetic prokaryotes the mechanisms of carbon fixation are more diverse. Here, carbon dioxide can be fixed by the Calvin – Benson cycle, a reversed citric acid cycle, or the carboxylation of acetyl-CoA. Prokaryotic chemoautotrophs also fix CO_2 through the Calvin – Benson cycle, but use energy from inorganic compounds to drive the reaction.

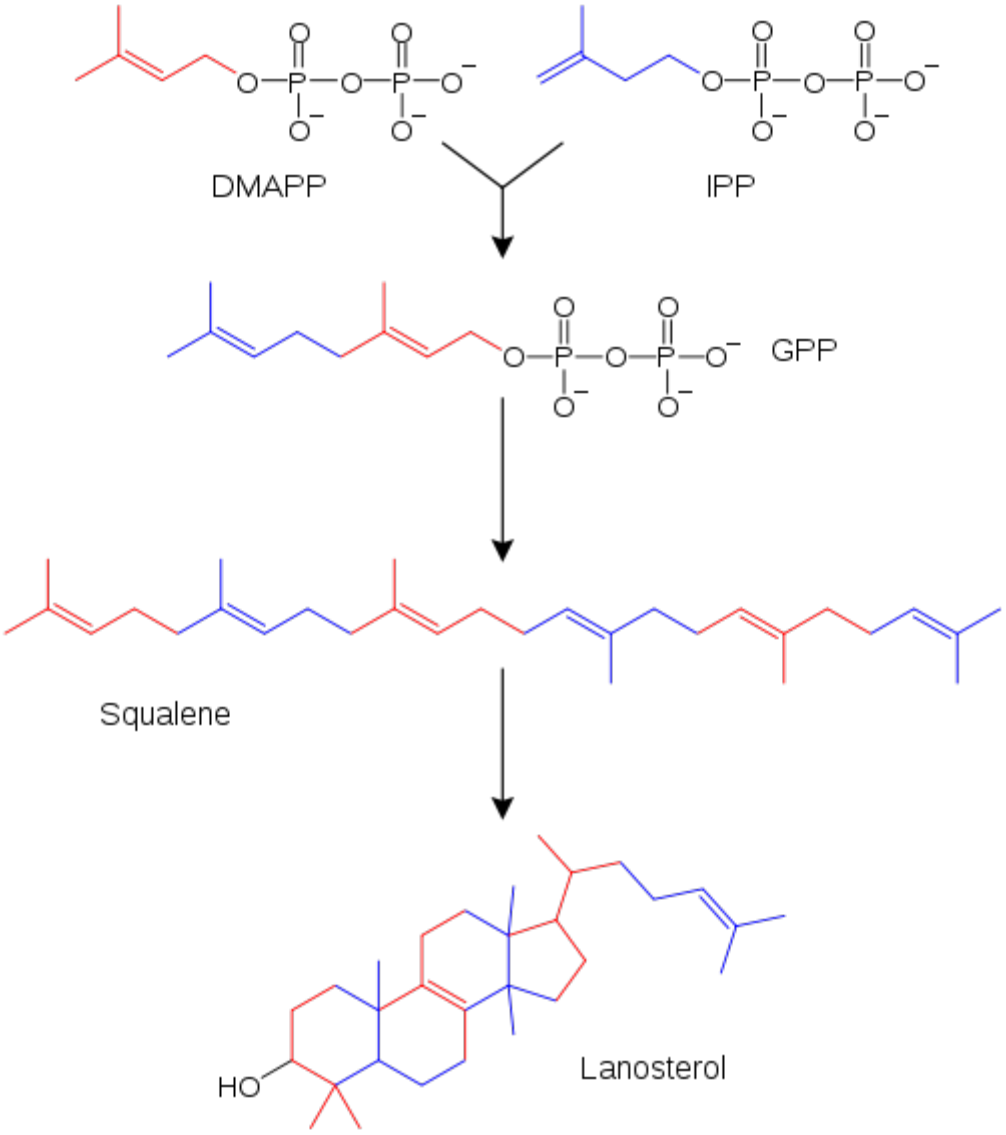
Carbohydrates and glycans

In carbohydrate anabolism, simple organic acids can be converted into monosaccharides such as glucose and then used to assemble polysaccharides such as starch. The generation of glucose from compounds like pyruvate, lactate, glycerol, glycerate 3-phosphate and amino acids is called gluconeogenesis. Gluconeogenesis converts pyruvate to glucose-6-phosphate through a series of intermediates, many of which are shared with glycolysis. However, this pathway is not simply glycolysis run in reverse, as several steps are catalyzed by non-glycolytic enzymes. This is important as it allows the formation and breakdown of glucose to be regulated separately and prevents both pathways from running simultaneously in a futile cycle.

Although fat is a common way of storing energy, in vertebrates such as humans the fatty acids in these stores cannot be converted to glucose through gluconeogenesis as these organisms cannot convert acetyl-CoA into pyruvate; plants do, but animals do not, have the necessary enzymatic machinery. As a result, after long-term starvation, vertebrates need to produce ketone bodies from fatty acids to replace glucose in tissues such as the brain that cannot metabolize fatty acids. In other organisms such as plants and bacteria, this metabolic problem is solved using the glyoxylate cycle, which bypasses the decarboxylation step in the citric acid cycle and allows the transformation of acetyl-CoA to oxaloacetate, where it can be used for the production of glucose.

Polysaccharides and glycans are made by the sequential addition of monosaccharides by glycosyltransferase from a reactive sugar-phosphate donor such as uridine diphosphate glucose (UDP-glucose) to an acceptor hydroxyl group on the growing polysaccharide. As any of the hydroxyl groups on the ring of the substrate can be acceptors, the polysaccharides produced can have straight or branched structures. The polysaccharides produced can have structural or metabolic functions themselves, or be transferred to lipids and proteins by enzymes called oligosaccharyltransferases.

Fatty acids, isoprenoids and steroids



Simplified version of the steroid synthesis pathway with the intermediates isopentenyl pyrophosphate (IPP), dimethylallyl pyrophosphate (DMAPP), geranyl pyrophosphate (GPP) and squalene shown. Some intermediates are omitted for clarity.

Fatty acids are made by fatty acid synthases that polymerize and then reduce acetyl-CoA units. The acyl chains in the fatty acids are extended by a cycle of reactions that add the actyl group, reduce it to an alcohol, dehydrate it to an alkene group and then reduce it again to an alkane group. The enzymes of fatty acid biosynthesis are divided into two groups, in animals and fungi all these fatty acid synthase reactions are carried out by a single multifunctional type I protein, while in plant plastids and bacteria separate type II enzymes perform each step in the pathway.

Terpenes and isoprenoids are a large class of lipids that include the carotenoids and form the largest class of plant natural products. These compounds are made by the assembly and modification of isoprene units donated from the reactive precursors isopentenyl pyrophosphate and dimethylallyl pyrophosphate. These precursors can be made in different ways. In animals and archaea, the mevalonate pathway produces these compounds from acetyl-CoA, while in plants and bacteria the non-mevalonate pathway uses pyruvate and glyceraldehyde 3-phosphate as substrates. One important reaction that uses these activated isoprene donors is steroid biosynthesis. Here, the isoprene units are joined together to make squalene and then folded up and formed into a set of rings to make lanosterol. Lanosterol can then be converted into other steroids such as cholesterol and ergosterol.

Proteins

Organisms vary in their ability to synthesize the 20 common amino acids. Most bacteria and plants can synthesize all twenty, but mammals can synthesize only eleven nonessential amino acids. Thus, nine essential amino acids must be obtained from food. All amino acids are synthesized from intermediates in glycolysis, the citric acid cycle, or the pentose phosphate pathway. Nitrogen is provided by glutamate and glutamine. Amino acid synthesis depends on the formation of the appropriate alpha-keto acid, which is then transaminated to form an amino acid.

Amino acids are made into proteins by being joined together in a chain by peptide bonds. Each different protein has a unique sequence of amino acid residues: this is its primary structure. Just as the letters of the alphabet can be combined to form an almost endless variety of words, amino acids can be linked in varying sequences to form a huge variety of proteins. Proteins are made from amino acids that have been activated by attachment to a transfer RNA molecule through an ester bond. This aminoacyl-tRNA precursor is produced in an ATP-dependent reaction carried out by an aminoacyl tRNA synthetase. This aminoacyl-tRNA is then a substrate for the ribosome, which joins the amino acid onto the elongating protein chain, using the sequence information in a messenger RNA.

Nucleotide synthesis and salvage

Nucleotides are made from amino acids, carbon dioxide and formic acid in pathways that require large amounts of metabolic energy. Consequently, most organisms have efficient systems to salvage preformed nucleotides. Purines are synthesized as nucleosides (bases attached to ribose). Both adenine and guanine are made from the precursor nucleoside inosine monophosphate, which is synthesized using atoms from the amino acids glycine, glutamine, and aspartic acid, as well as formate transferred from the coenzyme tetrahydrofolate. Pyrimidines, on the other hand, are synthesized from the base orotate, which is formed from glutamine and aspartate.

Xenobiotics and redox metabolism

All organisms are constantly exposed to compounds that they cannot use as foods and would be harmful if they accumulated in cells, as they have no metabolic function. These potentially damaging compounds are called xenobiotics. Xenobiotics such as synthetic drugs, natural poisons and antibiotics are detoxified by a set of xenobiotic-metabolizing enzymes. In humans, these include cytochrome P450 oxidases, UDP-glucuronosyltransferases, and glutathione *S*-transferases. This system of enzymes acts in three stages to firstly oxidize the xenobiotic (phase I) and then conjugate water-soluble groups onto the molecule (phase II). The modified water-soluble xenobiotic can then be pumped out of cells and in multicellular organisms may be further metabolized before being excreted (phase III). In ecology, these reactions are particularly important in microbial biodegradation of pollutants and the bioremediation of contaminated land and oil spills. Many of these microbial reactions are shared with multicellular organisms, but due to the incredible diversity of types of microbes these organisms are able to deal with a far wider range of xenobiotics than multicellular organisms, and can degrade even persistent organic pollutants such as organochloride compounds.

A related problem for aerobic organisms is oxidative stress. Here, processes including oxidative phosphorylation and the formation of disulfide bonds during protein folding produce reactive oxygen species such as hydrogen peroxide. These damaging oxidants are removed by antioxidant metabolites such as glutathione and enzymes such as catalases and peroxidases.

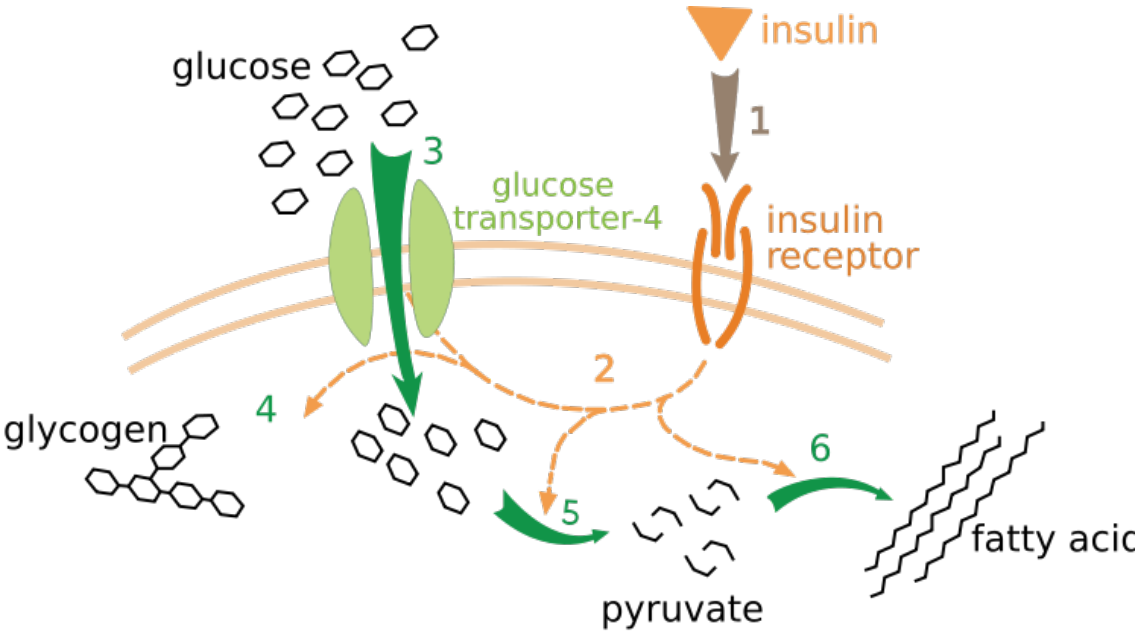
Thermodynamics of living organisms

Living organisms must obey the laws of thermodynamics, which describe the transfer of heat and work. The second law of thermodynamics states that in any closed system, the amount of entropy (disorder) will tend to increase. Although living organisms' amazing complexity appears to contradict this law, life is possible as all organisms are open systems that exchange matter and energy with their surroundings. Thus living systems are not in equilibrium, but instead are dissipative systems that maintain their state of high complexity by causing a larger increase in the entropy of their environments. The metabolism of a cell achieves this by coupling the spontaneous processes of catabolism to the non-spontaneous processes of anabolism. In thermodynamic terms, metabolism maintains order by creating disorder.

Regulation and control

As the environments of most organisms are constantly changing, the reactions of metabolism must be finely regulated to maintain a constant set of conditions within cells, a condition called homeostasis. Metabolic regulation also allows organisms to respond to signals and interact actively with their environments. Two closely linked concepts are important for understanding how metabolic pathways are controlled. Firstly, the *regulation* of an enzyme in a pathway is how its activity is increased and decreased in

response to signals. Secondly, the *control* exerted by this enzyme is the effect that these changes in its activity have on the overall rate of the pathway (the flux through the pathway). For example, an enzyme may show large changes in activity (*i.e.* it is highly regulated) but if these changes have little effect on the flux of a metabolic pathway, then this enzyme is not involved in the control of the pathway.



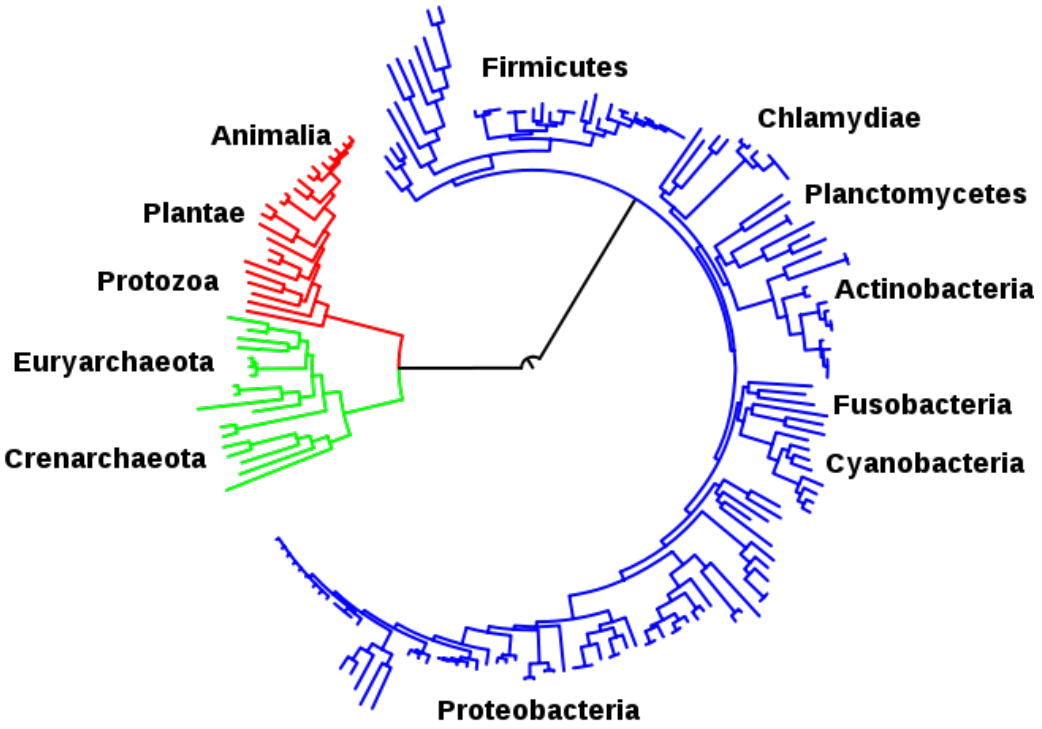
Effect of insulin on glucose uptake and metabolism. Insulin binds to its receptor (1) which in turn starts many protein activation cascades (2). These include: translocation of Glut-4 transporter to the plasma membrane and influx of glucose (3), glycogen synthesis (4), glycolysis (5) and fatty acid synthesis (6).

There are multiple levels of metabolic regulation. In intrinsic regulation, the metabolic pathway self-regulates to respond to changes in the levels of substrates or products; for example, a decrease in the amount of product can increase the flux through the pathway to compensate. This type of regulation often involves allosteric regulation of the activities of multiple enzymes in the pathway. Extrinsic control involves a cell in a multicellular organism changing its metabolism in response to signals from other cells. These signals are usually in the form of soluble messengers such as hormones and growth factors and are detected by specific receptors on the cell surface. These signals are then transmitted inside the cell by second messenger systems that often involved the phosphorylation of proteins.

A very well understood example of extrinsic control is the regulation of glucose metabolism by the hormone insulin. Insulin is produced in response to rises in blood glucose levels. Binding of the hormone to insulin receptors on cells then activates a cascade of protein kinases that cause the cells to take up glucose and convert it into storage molecules such as fatty acids and glycogen. The metabolism of glycogen is controlled by activity of phosphorylase, the enzyme that breaks down glycogen, and

glycogen synthase, the enzyme that makes it. These enzymes are regulated in a reciprocal fashion, with phosphorylation inhibiting glycogen synthase, but activating phosphorylase. Insulin causes glycogen synthesis by activating protein phosphatases and producing a decrease in the phosphorylation of these enzymes.

Evolution



Evolutionary tree showing the common ancestry of organisms from all three domains of life. Bacteria are colored blue, eukaryotes red, and archaea green. Relative positions of some of the phyla included are shown around the tree.

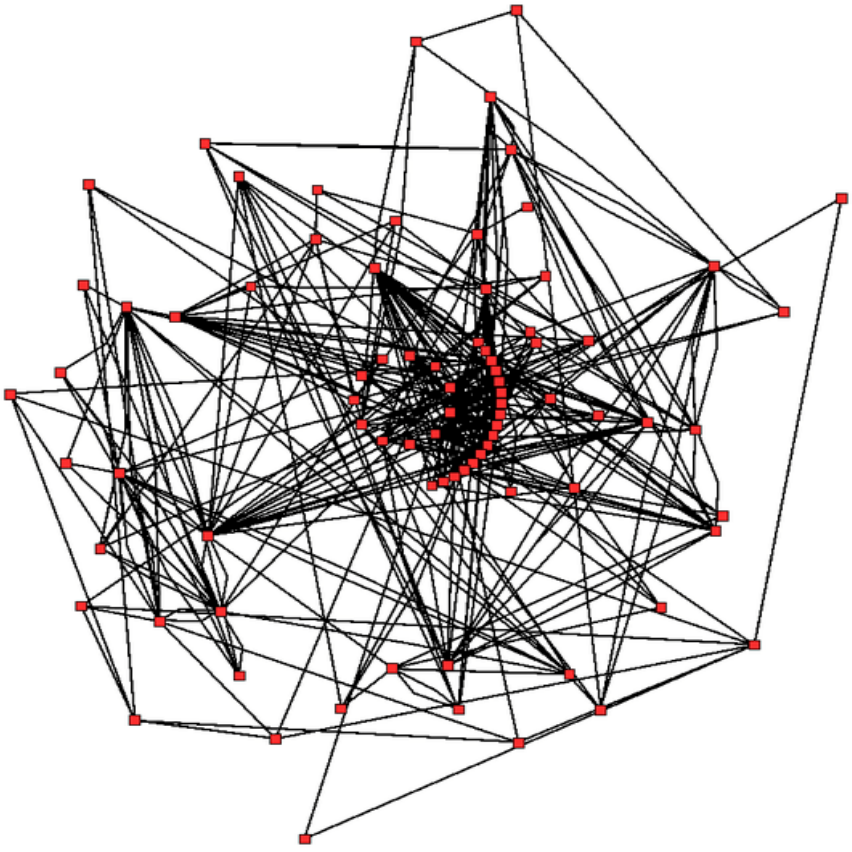
The central pathways of metabolism described above, such as glycolysis and the citric acid cycle, are present in all three domains of living things and were present in the last universal ancestor. This universal ancestral cell was prokaryotic and probably a methanogen that had extensive amino acid, nucleotide, carbohydrate and lipid metabolism. The retention of these ancient pathways during later evolution may be the result of these reactions being an optimal solution to their particular metabolic problems, with pathways such as glycolysis and the citric acid cycle producing their end products highly efficiently and in a minimal number of steps. The first pathways of enzyme-based metabolism may have been parts of purine nucleotide metabolism, with previous metabolic pathways being part of the ancient RNA world.

Many models have been proposed to describe the mechanisms by which novel metabolic pathways evolve. These include the sequential addition of novel enzymes to a short ancestral pathway, the duplication and then divergence of entire pathways as well as the

recruitment of pre-existing enzymes and their assembly into a novel reaction pathway. The relative importance of these mechanisms is unclear, but genomic studies have shown that enzymes in a pathway are likely to have a shared ancestry, suggesting that many pathways have evolved in a step-by-step fashion with novel functions being created from pre-existing steps in the pathway. An alternative model comes from studies that trace the evolution of proteins' structures in metabolic networks, this has suggested that enzymes are pervasively recruited, borrowing enzymes to perform similar functions in different metabolic pathways (evident in the MANET database) These recruitment processes result in an evolutionary enzymatic mosaic. A third possibility is that some parts of metabolism might exist as "modules" that can be reused in different pathways and perform similar functions on different molecules.

As well as the evolution of new metabolic pathways, evolution can also cause the loss of metabolic functions. For example, in some parasites metabolic processes that are not essential for survival are lost and preformed amino acids, nucleotides and carbohydrates may instead be scavenged from the host. Similar reduced metabolic capabilities are seen in endosymbiotic organisms.

Investigation and manipulation



Metabolic network of the *Arabidopsis thaliana* citric acid cycle. Enzymes and metabolites are shown as red squares and the interactions between them as black lines.

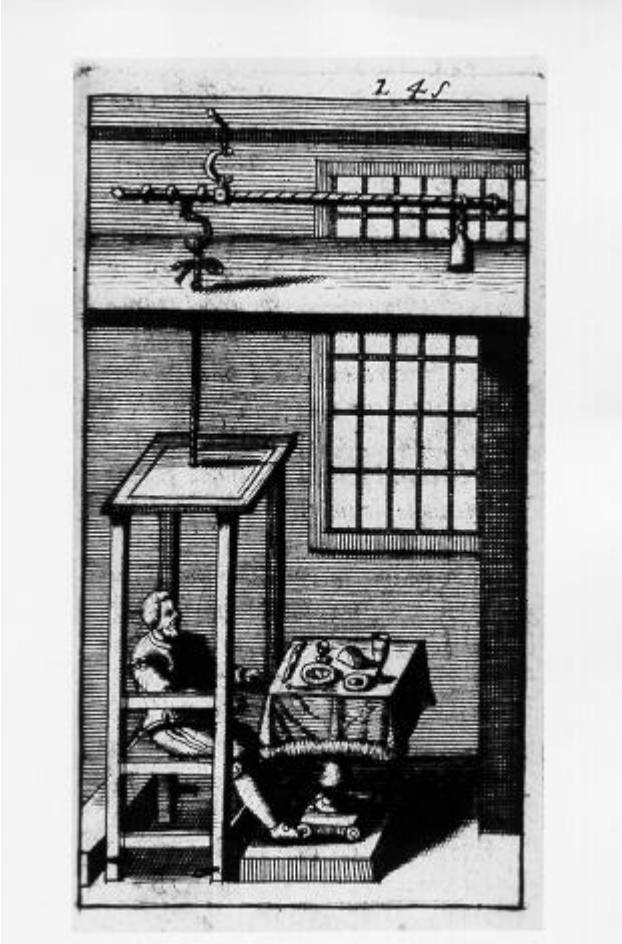
Classically, metabolism is studied by a reductionist approach that focuses on a single metabolic pathway. Particularly valuable is the use of radioactive tracers at the whole-organism, tissue and cellular levels, which define the paths from precursors to final products by identifying radioactively labelled intermediates and products. The enzymes that catalyze these chemical reactions can then be purified and their kinetics and responses to inhibitors investigated. A parallel approach is to identify the small molecules in a cell or tissue; the complete set of these molecules is called the metabolome. Overall, these studies give a good view of the structure and function of simple metabolic pathways, but are inadequate when applied to more complex systems such as the metabolism of a complete cell.

An idea of the complexity of the metabolic networks in cells that contain thousands of different enzymes is given by the figure showing the interactions between just 43 proteins and 40 metabolites to the right: the sequences of genomes provide lists containing anything up to 45,000 genes. However, it is now possible to use this genomic data to reconstruct complete networks of biochemical reactions and produce more holistic mathematical models that may explain and predict their behavior. These models are especially powerful when used to integrate the pathway and metabolite data obtained through classical methods with data on gene expression from proteomic and DNA microarray studies. Using these techniques, a model of human metabolism has now been produced, which will guide future drug discovery and biochemical research. These models are now being used in network analysis, to classify human diseases into groups that share common proteins or metabolites.

Bacterial metabolic networks seem to be a striking example of bow-tie organization, an architecture able to input a wide range of nutrients and produce a large variety of products and complex macromolecules using a relatively few intermediate common currencies.

A major technological application of this information is metabolic engineering. Here, organisms such as yeast, plants or bacteria are genetically modified to make them more useful in biotechnology and aid the production of drugs such as antibiotics or industrial chemicals such as 1,3-propanediol and shikimic acid. These genetic modifications usually aim to reduce the amount of energy used to produce the product, increase yields and reduce the production of wastes.

History



Santorio Santorio in his steelyard balance, from *Ars de statica medicina*, first published 1614

The term *metabolism* is derived from the Greek Μεταβολισμός – "Metabolismos" for "change", or "overthrow". The history of the scientific study of metabolism spans several centuries and has moved from examining whole animals in early studies, to examining individual metabolic reactions in modern biochemistry. The first controlled experiments in human metabolism were published by Santorio Santorio in 1614 in his book *Ars de statica medicina*. He described how he weighed himself before and after eating, sleep, working, sex, fasting, drinking, and excreting. He found that most of the food he took in was lost through what he called "insensible perspiration".

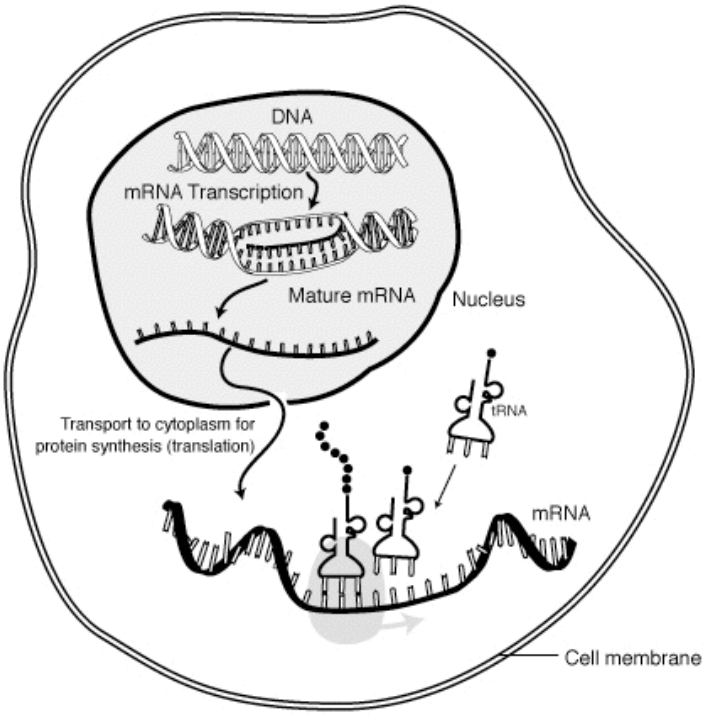
In these early studies, the mechanisms of these metabolic processes had not been identified and a vital force was thought to animate living tissue. In the 19th century, when studying the fermentation of sugar to alcohol by yeast, Louis Pasteur concluded that fermentation was catalyzed by substances within the yeast cells he called "ferments". He wrote that "alcoholic fermentation is an act correlated with the life and organization of

the yeast cells, not with the death or putrefaction of the cells." This discovery, along with the publication by Friedrich Wöhler in 1828 of the chemical synthesis of urea, proved that the organic compounds and chemical reactions found in cells were no different in principle than any other part of chemistry.

It was the discovery of enzymes at the beginning of the 20th century by Eduard Buchner that separated the study of the chemical reactions of metabolism from the biological study of cells, and marked the beginnings of biochemistry. The mass of biochemical knowledge grew rapidly throughout the early 20th century. One of the most prolific of these modern biochemists was Hans Krebs who made huge contributions to the study of metabolism. He discovered the urea cycle and later, working with Hans Kornberg, the citric acid cycle and the glyoxylate cycle. Modern biochemical research has been greatly aided by the development of new techniques such as chromatography, X-ray diffraction, NMR spectroscopy, radioisotopic labelling, electron microscopy and molecular dynamics simulations. These techniques have allowed the discovery and detailed analysis of the many molecules and metabolic pathways in cells.

Chapter- 5

Protein Biosynthesis



RNA is transcribed in the nucleus; once completely processed, it is transported to the cytoplasm and translated by the ribosome (not shown).

Protein synthesis is the process in which cells build proteins. The term is sometimes used to refer only to protein translation but more often it refers to a multi-step process, beginning with amino acid synthesis and transcription of nuclear DNA into messenger RNA, which is then used as input to translation.

The cistron DNA is transcribed into a variety of RNA intermediates. The last version is used as a template in synthesis of a polypeptide chain. Proteins can often be synthesized directly from genes by translating mRNA. When a protein needs to be available on short notice or in large quantities, a protein precursor is produced. A proprotein is an inactive

protein containing one or more inhibitory peptides that can be activated when the inhibitory sequence is removed by proteolysis during posttranslational modification. A preprotein is a form that contains a signal sequence (an N-terminal signal peptide) that specifies its insertion into or through membranes; i.e., targets them for secretion. The signal peptide is cleaved off in the endoplasmic reticulum. Preproproteins have both sequences (inhibitory and signal) still present.

For synthesis of protein, a succession of tRNA molecules charged with appropriate amino acids have to be brought together with an mRNA molecule and matched up by base-pairing through their anti-codons with each of its successive codons. The amino acids then have to be linked together to extend the growing protein chain, and the tRNAs, relieved of their burdens, have to be released. This whole complex of processes is carried out by a giant multimolecular machine, the ribosome, formed of two main chains of RNA, called ribosomal RNA (rRNA), and more than 50 different proteins. This molecular juggernaut latches onto the end of an mRNA molecule and then trundles along it, capturing loaded tRNA molecules and stitching together the amino acids they carry to form a new protein chain.

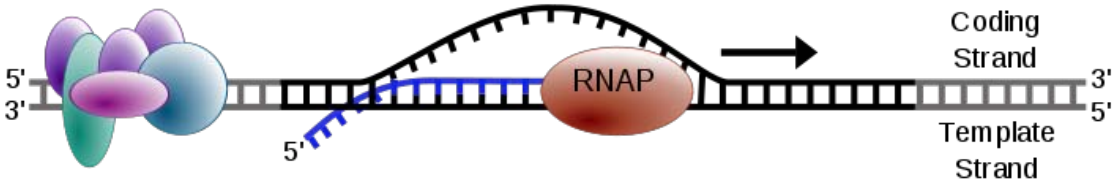
Protein biosynthesis, although very similar, is different for prokaryotes and eukaryotes.

Amino acid synthesis

Amino acids are the monomers that are polymerized to produce proteins. Amino acid synthesis is the set of biochemical processes (metabolic pathways) that build the amino acids from carbon sources like glucose.

Many organisms have the ability to synthesize only a subset of the amino acids they need. Adult humans, for example, need to obtain 10 of the 20 amino acids from their food.

Transcription



Simple diagram of transcription elongation

In transcription an mRNA chain is generated, with one strand of the DNA double helix in the genome as template. This strand is called the template strand. Transcription can be divided into 3 stages: Initiation, Elongation and Termination, each regulated by a large number of proteins such as transcription factors and coactivators that ensure the correct gene is transcribed.

The DNA strand is read in the 3' to 5' direction and the mRNA is transcribed in the 5' to 3' direction by the RNA polymerase.

Transcription occurs in the cell nucleus, where the DNA is held. The DNA structure of the cell is made up of two helixes made up of sugar and phosphate held together by the bases. The sugar and the phosphate are joined together by covalent bond. The DNA is "unzipped" by the enzyme helicase, leaving the single nucleotide chain open to be copied. RNA polymerase reads the DNA strand from 3 prime (3') end to the 5 prime (5') end, while it synthesizes a single strand of messenger RNA in the 5' to 3' direction. The general RNA structure is very similar to the DNA structure, but in RNA the nucleotide uracil takes the place that thymine occupies in DNA. The single strand of mRNA leaves the nucleus through nuclear pores, and migrates into the cytoplasm.

The first product of transcription differs in prokaryotic cells from that of eukaryotic cells, as in prokaryotic cells the product is mRNA, which needs no post-transcriptional modification, while in eukaryotic cells, the first product is called primary transcript, that needs post-transcriptional modification (capping with 7 methyl guanosine, tailing with a poly A tail) to give hnRNA (heterophil nuclear RNA). hnRNA then undergoes splicing of introns (non coding parts of the gene) via spliceosomes to produce the final mRNA.

Translation

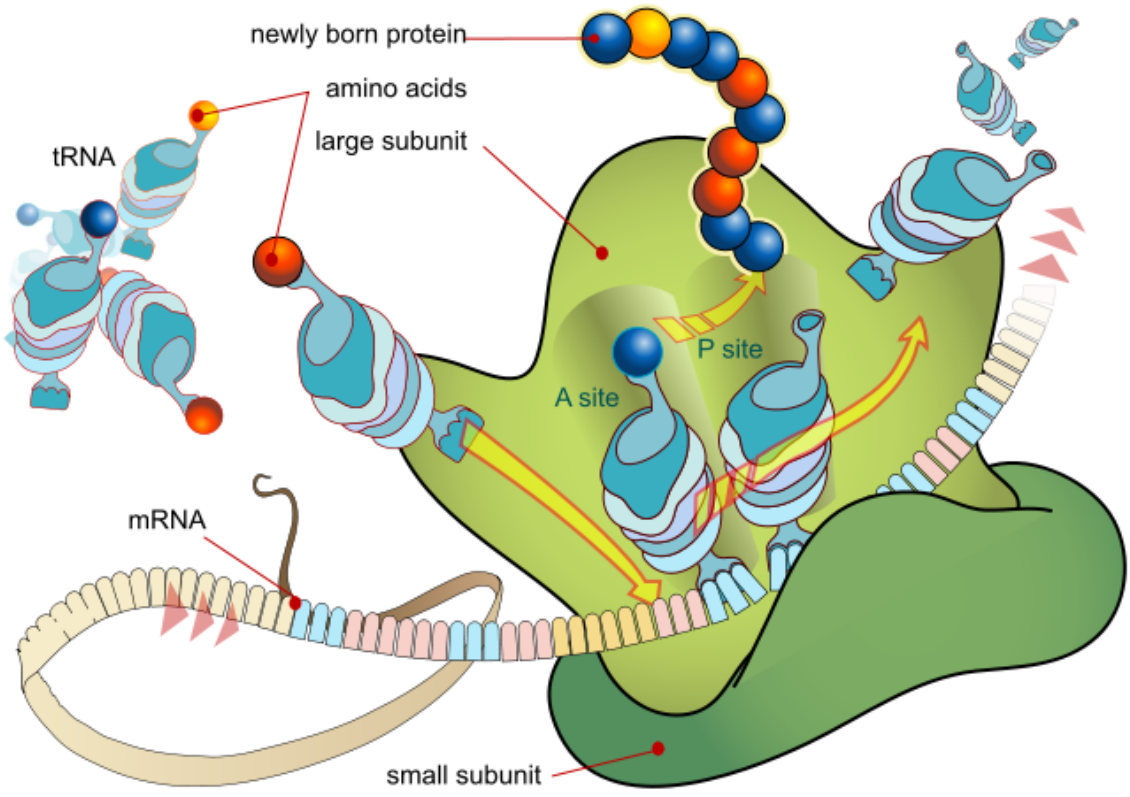


Diagram showing the translation of mRNA and the synthesis of proteins by a ribosome

The synthesis of proteins is known as translation. Translation occurs in the cytoplasm, where the ribosomes are located. Ribosomes are made of a small and large subunit that surround the mRNA. In translation, messenger RNA (mRNA) is decoded to produce a specific polypeptide according to the rules specified by the trinucleotide genetic code. This uses an mRNA sequence as a template to guide the synthesis of a chain of amino acids that form a protein. Translation proceeds in four phases: activation, initiation, elongation, and termination (all describing the growth of the amino acid chain, or polypeptide that is the product of translation).

In activation, the correct amino acid (AA) is joined to the correct transfer RNA (tRNA). While this is not technically a step in translation, it is required for translation to proceed. The AA is joined by its carboxyl group to the 3' OH of the tRNA by an ester bond. When the tRNA has an amino acid linked to it, it is termed "charged". Initiation involves the small subunit of the ribosome binding to 5' end of mRNA with the help of initiation factors (IF), other proteins that assist the process. Elongation occurs when the next aminoacyl-tRNA (charged tRNA) in line binds to the ribosome along with GTP and an elongation factor. Termination of the polypeptide happens when the A site of the ribosome faces a stop codon (UAA, UAG, or UGA). When this happens, no tRNA can recognize it, but releasing factor can recognize nonsense codons and causes the release of the polypeptide chain. The capacity of disabling or inhibiting translation in protein biosynthesis is used by antibiotics such as: anisomycin, cycloheximide, chloramphenicol, tetracycline, streptomycin, erythromycin, puromycin etc.

Translation is the process of converting the mRNA codon sequences into an amino acid polypeptide chain.

1.Amino acid activation

2.Initiation - A ribosome attaches to the mRNA and starts to code at the FMet codon (usually AUG, sometimes GUG or UUG).

3.Elongation - tRNA brings the corresponding amino acid (which has an anticodon that identifies the amino acid as the corresponding molecule to a codon) to each codon as the ribosome moves down the mRNA strand.

4.Termination - Reading of the final mRNA codon (aka the STOP codon), which ends the synthesis of the peptide chain and releases it.

Events following protein translation

The events following biosynthesis include post-translational modification and protein folding. During and after synthesis, polypeptide chains often fold to assume, so called, native secondary and tertiary structures. This is known as *protein folding*.

Many proteins undergo *post-translational modification*. This may include the formation of disulfide bridges or attachment of any of a number of biochemical functional groups, such as acetate, phosphate, various lipids and carbohydrates. Enzymes may also remove one or more amino acids from the leading (amino) end of the polypeptide chain, leaving a protein consisting of two polypeptide chains connected by disulfide bonds.

In general, protein molecules are believed to be modified by small chemical groups, post-translationally. Chemical modifications such as, phosphorylation of serine / threonine, acetylation or methylation of lysine, hydroxylation of proline / lysine, formylation of glycine , glycosylation of serine / threonine / asparagine, acylation of cysteine , myristoylation of glycine , biotinylation of lysine, ubiquitination , etc. on proteins is a very important issue in relation to properly understanding the biological functions of a given protein. These much-studied post-translational modifications have become well-established with the discovery of the respective enzymes (kinases for phosphorylation, acetylases for acetylation, methyl transferases for methylation, etc.), which carry on the chemical modifications on the specific amino acid residues. All of these modifications are still believed to have happened as post-translational events. There is no study yet on when actually one particular modification occurs on a given amino acid residue in a given protein. Does it happen when the protein is already formed, or when the amino acid chain is being synthesized, or before the translation of the primary chain has begun?

Since these chemical modifications are related to the biological functions of a protein, it is easy to think that these chemical modifications have happened to the whole protein molecule, after the protein primary chain is fully synthesized; but, if that is the case, we have to consider the fact that the primary chains get folded instantly, (in a similar way as the newly synthesized DNA strands form helixes), to attain its compact-globular conformation ; As most of the primary chains are fairly long [a 5Kd protein may have 40-45 amino acid residues in its primary chain], it is likely that the newly formed amino acid chain tries to remain intact by folding, thereby avoiding its breakdown via lots of proteases present within the cytoplasm. And, no capping event to protect the N-terminal end of the primary sequence (similar to 5' m-RNA capping to protect m-RNAs) is ever discovered for protein primary structure. So, by folding mechanism, the primary chain, perhaps, avoids the protease attacks . However, once it gets folded, it may be very difficult for the respective enzyme molecule to find out the particular aa residue from the complexity of that compactly folded conformation. In addition, it can be clearly imagined that this enzymatic modification/reaction on a given amino acid requires presence and association of the appropriate enzyme, necessary cofactors, etc. This association is much easier to occur when the amino acid residues in the primary structure are readily available for binding; in other words, it is much more difficult for the enzyme molecules to find and to bind to its substrate amino acid residue in a mature protein molecule after its three-dimensional conformation been attained.

So one can think that the modifications can happen while the primary chain of the protein is being synthesized during the translation process on the m-RNA strand; the amino acid residues on the primary chain can be modified instantly and enzymatically by kinases,

acetylases, hydroxylases, methyltransferases, etc. to initiate proper folding for protection in order to avoid degradation by proteases, thereby gaining the globular form.

Also, the reader can imagine another scenario in which, while the free amino acid molecules are formed within a cell and become available, they [in that free state] may be modified enzymatically before taking the ride to the translational event; this means that, while the primary chain is being synthesized, the pre-modified amino acid molecules are ready to be engaged. This way, as the primary chain is getting synthesized, it does not have to be modified by the modifying enzymes anymore, and can fold itself instantaneously without concern of degradation. This also arises new thoughts that (i) phosphate, acetyl, methyl, biotinyl, acyl, etc. groups may have the ability to inhibit protease actions on the primary chains; (ii) most or all of the amino acid residues get modified by small chemical groups (so far, only some chemical groups are known).

It is still not fully known exactly when and how the actual modification of a given amino acid residue occurs, at which stage of synthesis, within a protein molecule.

Once the chemically-modified, protease-insensitive, intact protein molecule is generated, it must perform its biological function, which requires its being activated. The activation is probably done by a second set of enzymatic reactions when these chemical groups are removed from the aa residues (or added back). So, the second type of post-translational modifications are the opposite reactions of the above-described type, which are dephosphorylation by phosphatases or phosphoryl transferases, deacetylation by deacetylases or acetyltransferases, demethylation by demethylases or methyl-transferases, ubiquitination, SuMoylation, glycosylation, biotinylation, etc. These are taking place on the whole protein molecule toward generating its activated form to execute a particular function or toward its deactivation followed by its total degradation. As the chemical groups are removed (or added), it is quite clear that the proteins can go through different states of structural/conformational change. Thus, after translation, a protein can change its conformation dynamically while the chemical groups are removed or added enzymatically; these conformational changes in the protein structure help the protein to proceed through its lifecycle, until it is ubiquitinated for its total degradation.

Chapter- 6

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

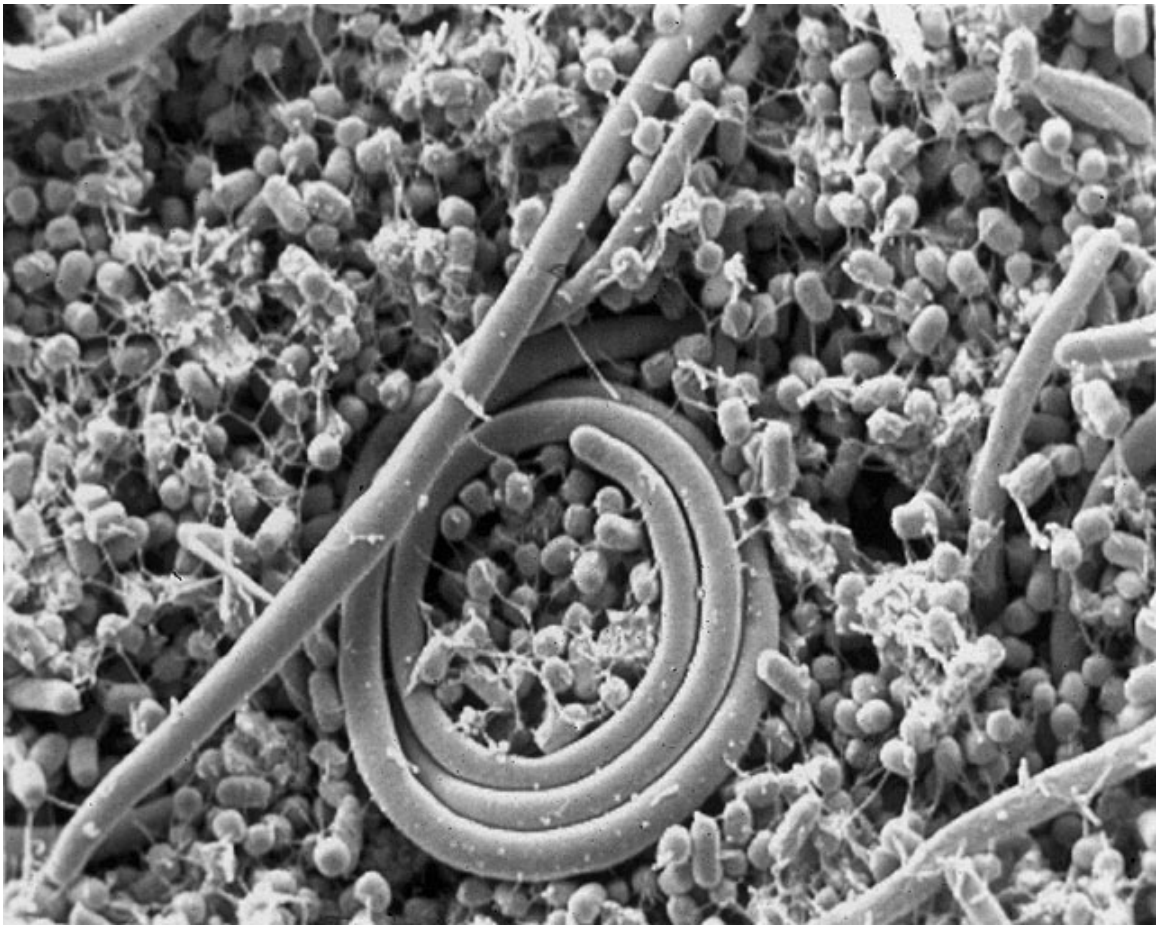


Figure 1. Example of signaling between bacteria. Salmonella enteritidis uses acyl-homoserine lactone for Quorum sensing (see: Inter-Bacterial Communication)

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

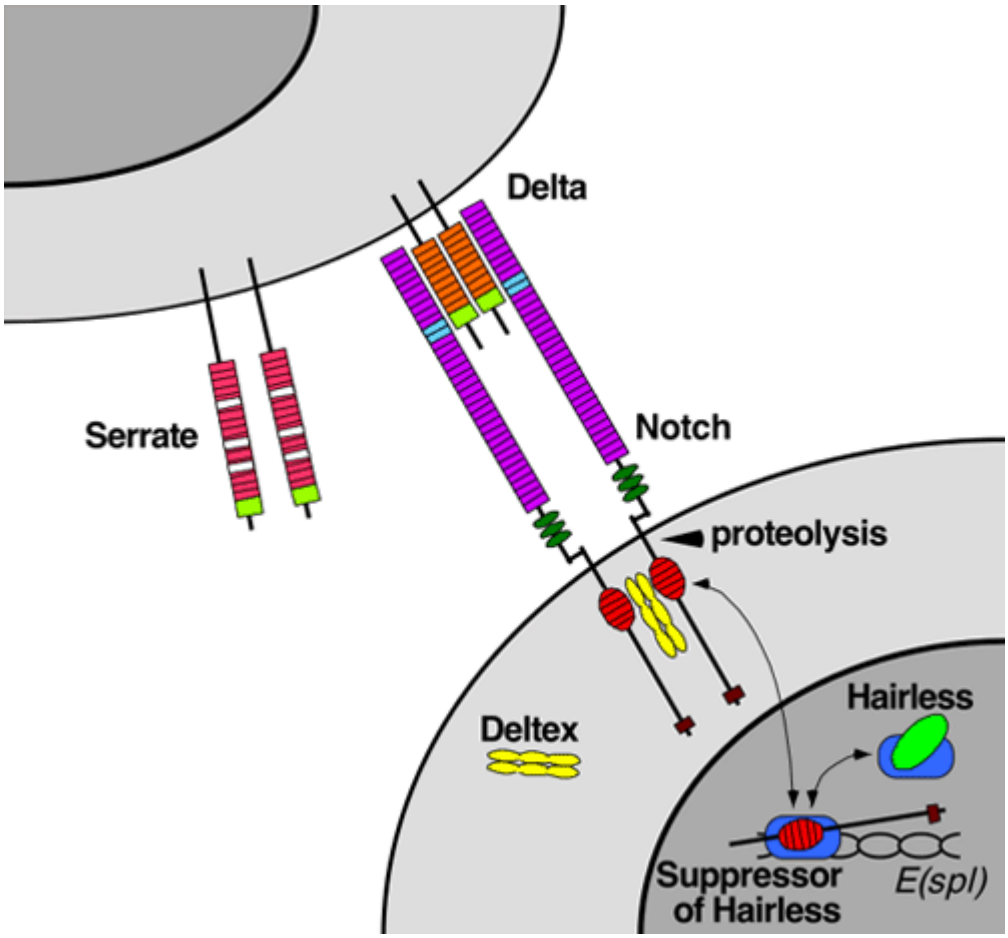


Figure 2. Notch-mediated juxtacrine signal between adjacent cells.

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

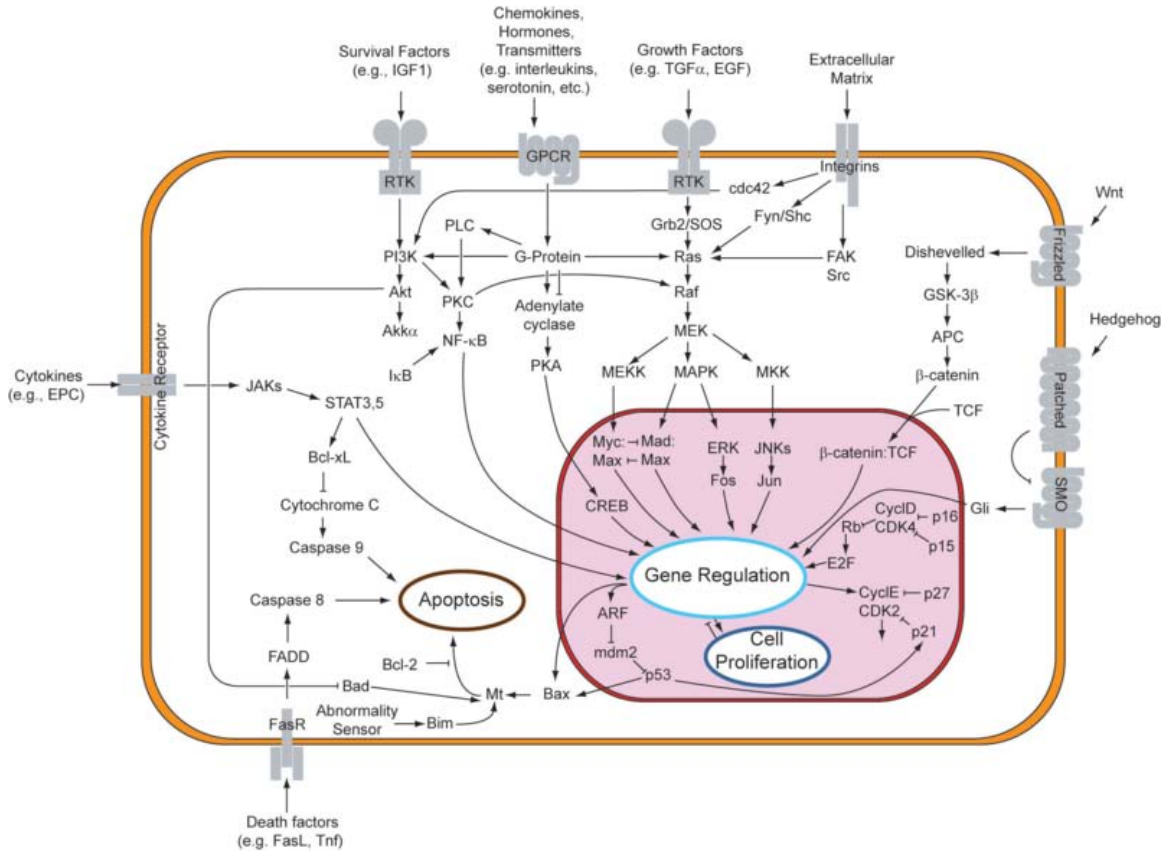
Cells receive information from their environment through a class of proteins known as receptors. Notch is a cell surface protein that functions as a receptor. Animals have a small set of genes that code for signaling proteins that interact specifically with Notch receptors and stimulate a response in 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.

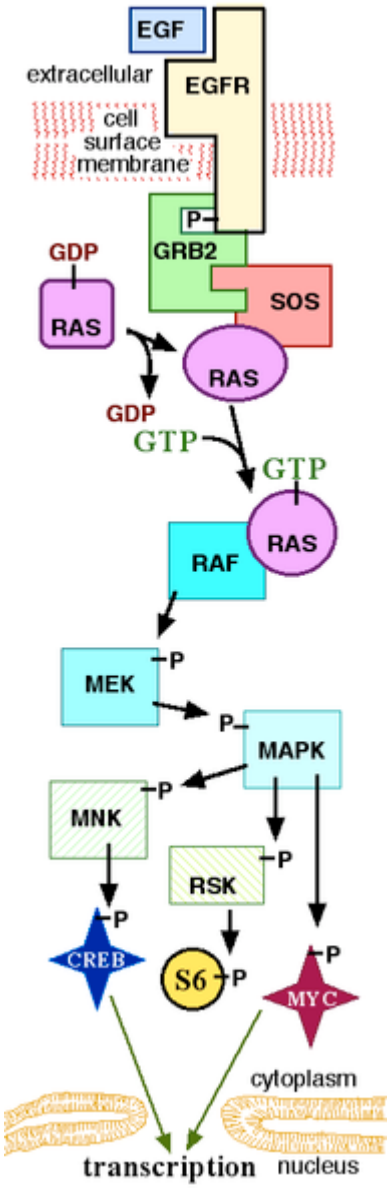


Figure 3. Diagram showing key components of a signal transduction pathway.

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

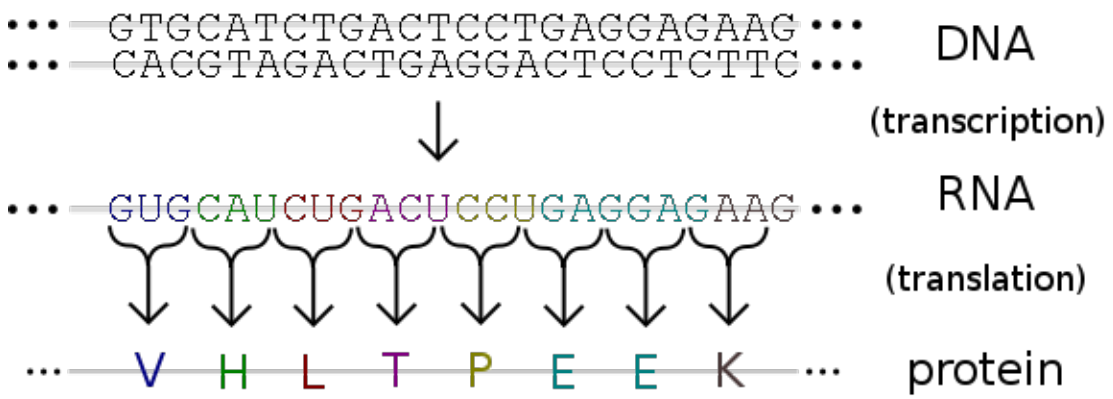
Within endocrinology (the study of intercellular signalling in animals) and the endocrine system, intercellular signalling is subdivided into the following classifications:

- *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- 7

Gene Expression



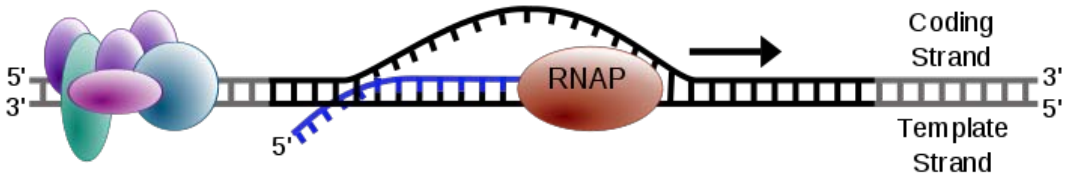
Genes are expressed by being transcribed into RNA, and this transcript may then be translated into protein.

Gene expression is the process by which information from a gene is used in the synthesis of a functional gene product. These products are often proteins, but in non-protein coding genes such as ribosomal RNA (rRNA) genes or transfer RNA (tRNA) genes, the product is a functional RNA. The process of gene expression is used by all known life - eukaryotes (including multicellular organisms), prokaryotes (bacteria and archaea) and viruses - to generate the macromolecular machinery for life. Several steps in the gene expression process may be modulated, including the transcription, RNA splicing, translation, and post-translational modification of a protein. Gene regulation gives the cell control over structure and function, and is the basis for cellular differentiation, morphogenesis and the versatility and adaptability of any organism. Gene regulation may also serve as a substrate for evolutionary change, since control of the timing, location, and amount of gene expression can have a profound effect on the functions (actions) of the gene in a cell or in a multicellular organism.

In genetics, gene expression is the most fundamental level at which genotype gives rise to the phenotype. The genetic code stored in DNA in form of nucleotide sequence is "interpreted" by gene expression, and the properties of the expression products give rise to the organism's phenotype.

Mechanism

Transcription



The process of transcription is carried out by RNA polymerase (RNAP), uses DNA (black) as a template and produces RNA (blue).

The gene itself is typically a long stretch of DNA which carries genetic information encoded by genetic code. Every molecule of DNA consists of two strands, each of them having 5' and 3' ends oriented in anti-parallel direction. The coding strand contains the genetic information while template strand (non-coding strand) serves as a blueprint for the production of RNA. The production of RNA copies of the DNA is called transcription, and is performed by RNA polymerase, which adds one RNA nucleotide at a time to a growing RNA strand. This RNA is complementary to the template 3' → 5' DNA strand, which is itself complementary to the coding 5' → 3' DNA strand. Therefore, the resulting 5' → 3' RNA strand is identical to the coding DNA strand with the exception that thymines (T) are replaced with uracils (U) in the RNA. A coding DNA strand reading "ATG" is transcribed as "AUG" in RNA.

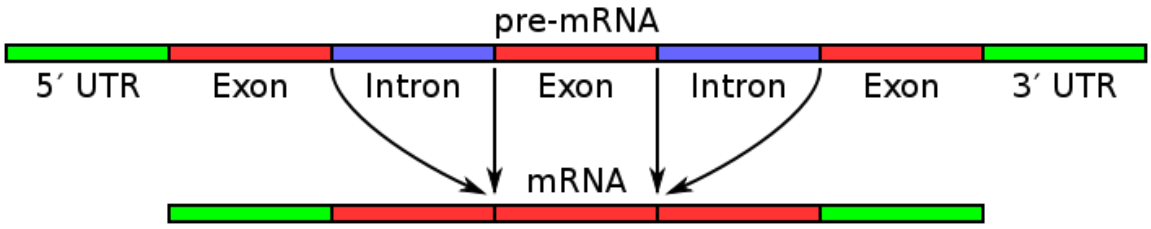
Transcription in prokaryotes is carried out by a single type of RNA polymerase, which needs DNA sequence called Pribnow box and sigma factor (σ factor) to start transcription. In eukaryotes, the transcription is done by three types of RNA polymerases, each of them needs special DNA sequence called promoter and a set of DNA-binding proteins - transcription factors to initiate the process. RNA polymerase I is responsible for transcription of rRNA genes, while RNA polymerase II transcribes all protein-coding genes but also some non-coding RNAs (e.g. snRNAs, snoRNAs or long non-coding RNAs) as well. It contains special part called C-terminal domain (CTD) that is rich of serines, which after being phosphorylated accumulate factors necessary for RNA modification and maturation. RNA polymerase III transcribes 5S rRNA and tRNA genes but also some small non-coding RNA genes (e.g. 7SK). Transcription ends on a special sequence called terminator.

RNA processing

While transcription of prokaryotic protein-coding genes creates messenger RNA (mRNA) which is ready for translation, transcription of eukaryotic genes leaves a primary transcript of RNA (pre-mRNA), which fist has to undergo series of modification to become a mature mRNA.

These include 5' *capping*, which is set of enzymatic reactions that add 7-methylguanosine (m^7G) to the 5' end of pre-mRNA and thus protect the RNA from degradation by exonucleases. The m^7G cap is then bound by cap binding complex heterodimer (CBC20/CBC80) which aids in mRNA export to cytoplasm and also protect the RNA from decapping.

Another modification is 3' *clevage and polyadenylation*. They occur if polyadenylation signal sequence (5'- AAUAAA-3') is present in pre-mRNA,which is usually between protein-coding sequence and terminator. The pre-mRNA is first cleaved and then a series of ~200 adenines (A) are added to form poly(A) tail which protects the RNA from degradation. Poly(A) tail is bound by multiple poly(A)-binding proteins (PABP) necessary for mRNA export and translation re-iniciation.



Simple illustration of exons and introns in pre-mRNA and the formation of mature mRNA by splicing. The UTRs are non-coding parts of exons at the ends of the mRNA.

Very important modification of eukaryotic pre-mRNA is *RNA splicing*. Majority of eukaryotic pre-mRNAs consist of alternating segments called exons and introns. During the process of splicing, RNA-protein catalytical complex known as spliceosome, catalyze two transesterification reactions, which remove intron and release it in form of lariat structure and then splice neighbouring exons together. In certain cases, some introns or exons can be either removed or retained in mature mRNA. This so-called alternative splicing creates series of different transcripts originating from a single gene. Because these transcripts can be potentially translated into different proteins, splicing extends the complexity of eukaryotic gene expression.

Extensive RNA processing may be an evolutionary advantage made possible by the nucleus of eukaryotes. In prokaryotes transcription and translation happen together whilst in eukaryotes the nuclear membrane separates the two processes giving time for RNA processing to occur.

non-coding RNA maturation

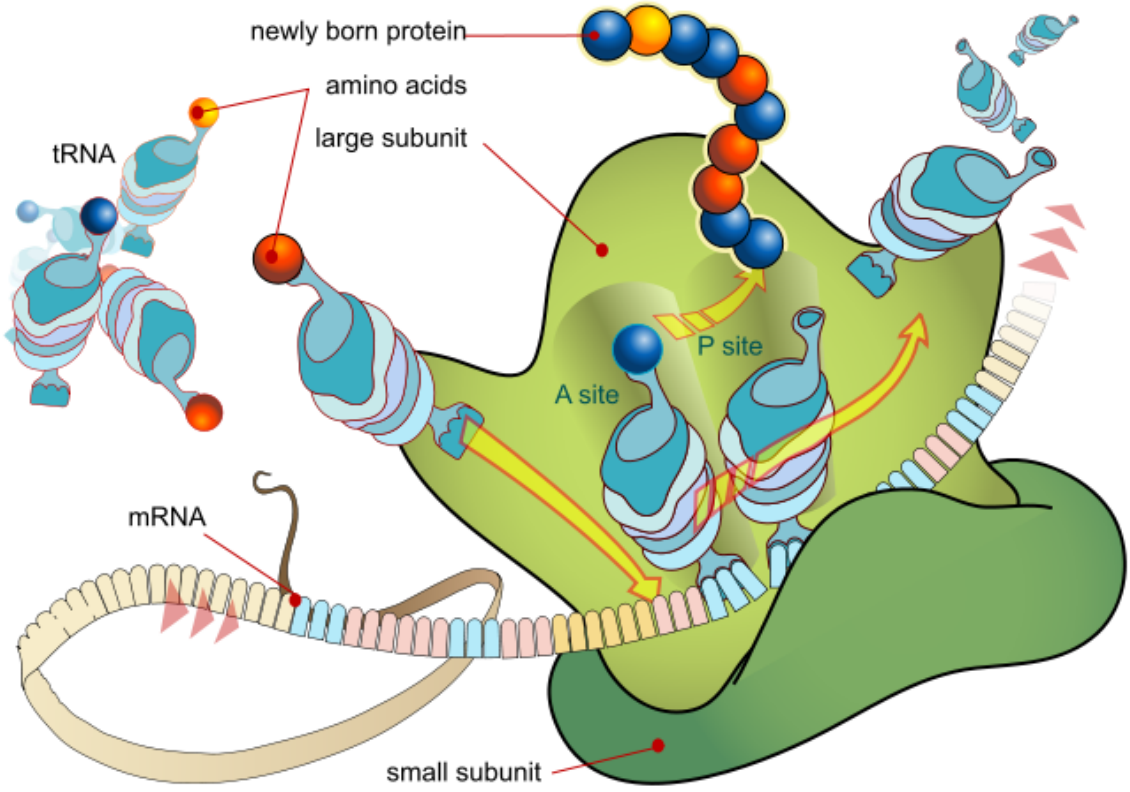
In most organisms non-coding genes (ncRNA) are transcribed as precursors which undergo further processing. In the case of ribosomal RNAs (rRNA), they are often transcribed as a pre-rRNA which contains one or more rRNAs, the pre-rRNA is cleaved and modified (2′-O-methylation and pseudouridine formation) at a specific sites by approximately 150 different small nucleolus-restricted RNA species, called snoRNAs. SnoRNAs associate with proteins, forming snoRNPs. While snoRNA part basepair with

the target RNA and thus position the modification to precise site, the protein part performs the catalytical reaction. In eukaryotes, in particular a snoRNP, called RNase MRP cleaves the 45S pre-rRNA into the 28S, 5.8S, and 18S rRNAs. The rRNA and RNA processing factors form large aggregates called the nucleolus.
In the case of transfer RNA (tRNA), for example, the 5' sequence is removed by RNase P, whereas the 3' end is removed by the tRNase Z enzyme and the non-templated 3' CCA tail is added by a nucleotidyl transferase. In the case of micro RNA (miRNA), miRNAs are first transcribed as primary transcripts or pri-miRNA with a cap and poly-A tail and processed to short, 70-nucleotide stem-loop structures known as pre-miRNA in the cell nucleus by the enzymes Drosha and Pasha. After being exported, it is then processed to mature miRNAs in the cytoplasm by interaction with the endonuclease Dicer, which also initiates the formation of the RNA-induced silencing complex (RISC), composed of the Argonaute protein. Even snRNAs and snoRNAs themselves undergo series of modification before they become part of functional RNP complex. This is done either in the nucleoplasm or in the specialized compartments called Cajal bodies. Their bases are methylated or pseudouridinilated by a group of small Cajal body-specific RNAs (scaRNAs) which are structurally similar to snoRNAs.

RNA export

In eukaryotes most mature RNA must be exported to the cytoplasm from the nucleus. While some RNAs function in the nucleus, many RNAs are transported through the nuclear pores and into the cytosol. Notably this includes all RNA types involved in protein synthesis. In some cases RNAs are additionally transported to a specific part of the cytoplasm, such as a synapse; they are then towed by motor proteins that bind through linker proteins to specific sequences (called "zipcodes") on the RNA.

Translation



During the translation, tRNA charged with amino acid enters the ribosome and aligns with the correct mRNA triplet. Ribosome then adds amino acid to growing protein chain.

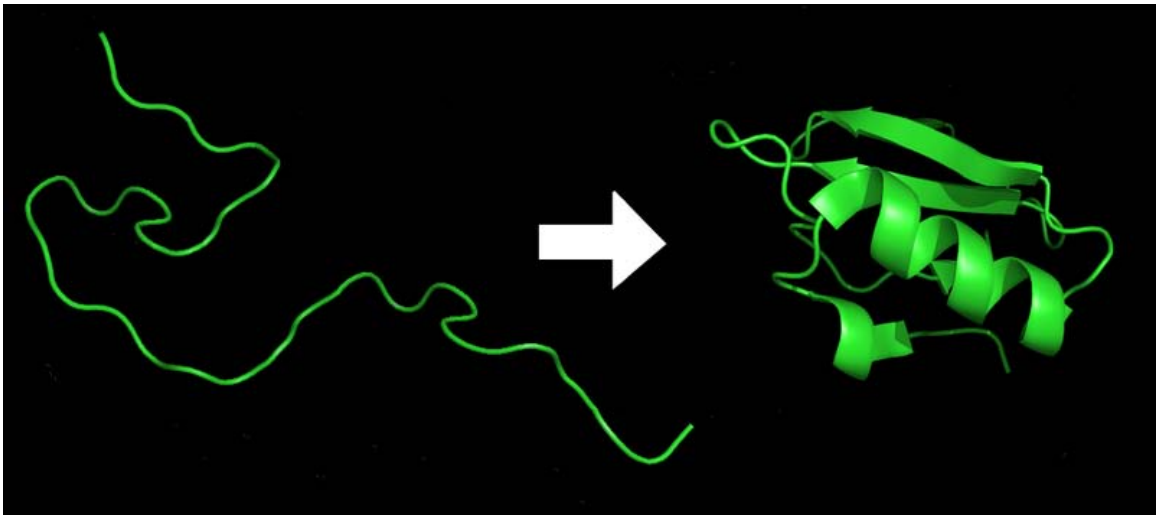
For some RNA (non-coding RNA) the mature RNA is the final gene product. In the case of messenger RNA (mRNA) the RNA is an information carrier coding for the synthesis of one or more proteins. mRNA carrying a single protein sequence (common in eukaryotes) is monocistronic whilst mRNA carrying multiple protein sequences (common in prokaryotes) is known as polycistronic.

Every mRNA consists of three parts - 5' untranslated region (5'UTR), protein-coding region or open reading frame (ORF) and 3' untranslated region (3'UTR). Coding region carries information for protein synthesis encoded by genetic code into form of triplets. Each triplet of nucleotides of the coding region is called codon and corresponds to a binding site complementary to an anticodon triplet in transfer RNA. Transfer RNAs with the same anticodon sequence always carry identical type of amino acid. Amino acids are then chained together by the ribosome according to order of triplets in the coding region. The ribosome helps transfer RNA to bind to messenger RNA and takes the amino acid from each transfer RNA and makes a structure-less protein out of it.

In prokaryotes translation generally occurs at the point of transcription (co-transcriptionally), often using a messenger RNA which is still in the process of being

created. In eukaryotes translation can occur in a variety of regions of the cell depending on where the protein being written is supposed to be. Major locations are the cytoplasm for soluble cytoplasmic proteins and the membrane of endoplasmic reticulum for proteins which are for export from the cell or insertion into a cell membrane. Proteins which are supposed to be expressed at the endoplasmic reticulum are recognised part-way through the translation process. This is governed by the signal recognition particle - a protein which binds to the ribosome and directs it to the endoplasmic reticulum when it finds a signal sequence on the growing (nascent) amino acid chain.

Folding



Protein before (left) and after (right) folding.

The polypeptide folds into its characteristic and functional three-dimensional structure from random coil. Each protein exists as an unfolded polypeptide or random coil when translated from a sequence of mRNA to a linear chain of amino acids. This polypeptide lacks any developed three-dimensional structure (the left hand side of the neighboring figure). Amino acids interact with each other to produce a well-defined three-dimensional structure, the folded protein (the right hand side of the figure), known as the native state. The resulting three-dimensional structure is determined by the amino acid sequence (Anfinsen's dogma).

The correct three-dimensional structure is essential to function, although some parts of functional proteins may remain unfolded Failure to fold into the intended shape usually produces inactive proteins with different properties including toxic prions. Several neurodegenerative and other diseases are believed to result from the accumulation of *misfolded* (incorrectly folded) proteins. Many allergies are caused by the folding of the proteins, for the immune system does not produce antibodies for certain protein structures.

Enzymes called chaperones assist the newly formed protein to attain (fold into) the 3-dimensional structure it needs to function. Similarly, RNA chaperones help RNAs attain

their functional shapes. Assisting protein folding is one of the main roles of the endoplasmic reticulum in eukaryotes.

Protein transport

Many proteins are destined for other parts of the cell than the cytosol and a wide range of signalling sequences are used to direct proteins to where they are supposed to be. In prokaryotes this is normally a simple process due to limited compartmentalisation of the cell. However in eukaryotes there is a great variety of different targeting processes to ensure the protein arrives at the correct organelle.

Not all proteins remain within the cell and many are exported, for example digestive enzymes, hormones and extracellular matrix proteins. In eukaryotes the export pathway is well developed and the main mechanism for the export of these proteins is translocation to the endoplasmic reticulum, followed by transport via the Golgi apparatus.

Regulation of gene expression

The patchy colours of a tortoiseshell cat are the result of different levels of expression of pigmentation genes in different areas of the skin.

Regulation of gene expression refers to the control of the amount and timing of appearance of the functional product of a gene. Control of expression is vital to allow a cell to produce the gene products it needs when it needs them; in turn this gives cells the flexibility to adapt to a variable environment, external signals, damage to the cell, etc. Some simple examples of where gene expression is important are:

- Control of Insulin expression so it gives a signal for blood glucose regulation
- X chromosome inactivation in female mammals to prevent an "overdose" of the genes it contains.
- Cyclin expression levels control progression through the eukaryotic cell cycle

More generally gene regulation gives the cell control over all structure and function, and is the basis for cellular differentiation, morphogenesis and the versatility and adaptability of any organism.

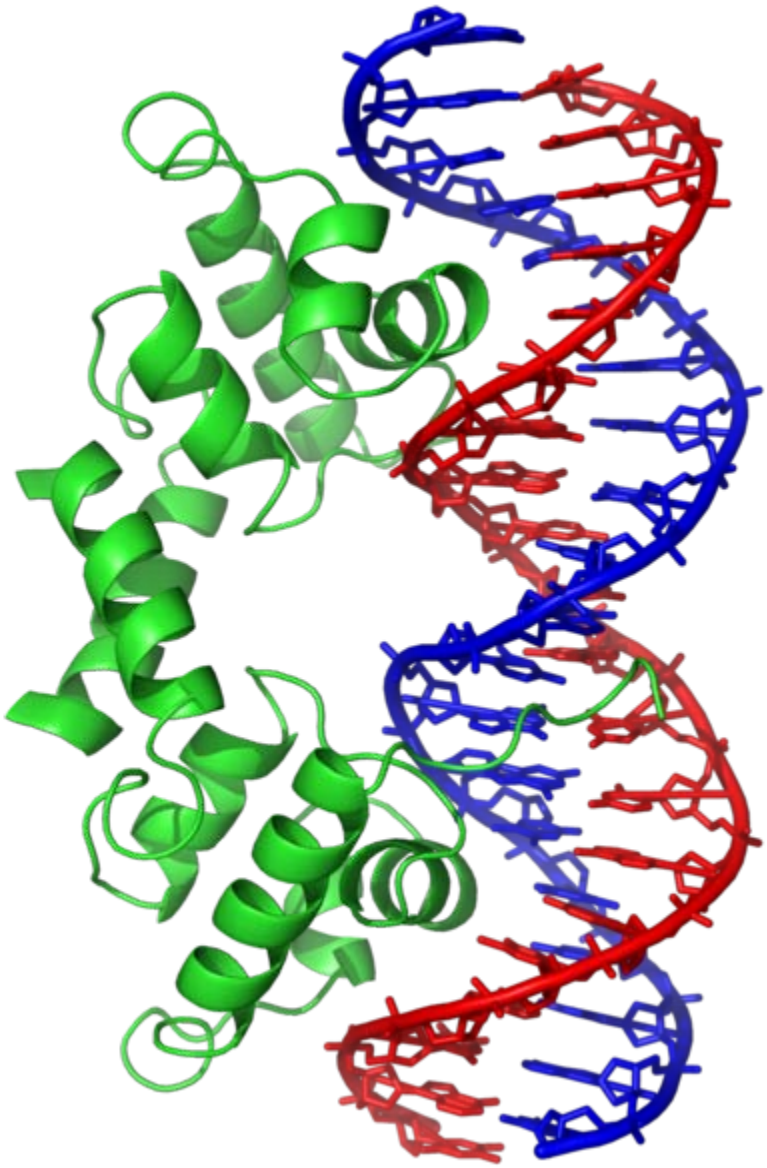
Any step of gene expression may be modulated, from the DNA-RNA transcription step to post-translational modification of a protein. The stability of the final gene product, whether it is RNA or protein, also contributes to the expression level of the gene - an unstable product results in a low expression level. In general gene expression is regulated through changes in the number and type of interactions between molecules that collectively influence transcription of DNA and translation of RNA.

Numerous terms are used to describe types of genes depending on how they are regulated, these include:

- A *constitutive gene* is a gene that is transcribed continually compared to a facultative gene which is only transcribed when needed.
- A *housekeeping gene* is typically a constitutive gene that is transcribed at a relatively constant level. The housekeeping gene's products are typically needed for maintenance of the cell. It is generally assumed that their expression is unaffected by experimental conditions. Examples include actin, GAPDH and ubiquitin.
- A *facultative gene* is a gene which is only transcribed when needed compared to a constitutive gene.
- An *inducible gene* is a gene whose expression is either responsive to environmental change or dependent on the position in the cell cycle.

Transcriptional regulation

Regulation of transcription can be broken down into three main routes of influence; genetic (direct interaction of a control factor with the gene), modulation (interaction of a control factor with the transcription machinery) and epigenetic (non-sequence changes in DNA structure which influence transcription).



The lambda repressor transcription factor (green) binds as a dimer to major groove of DNA target (red and blue) and disables initiation of transcription. From PDB 1LMB.

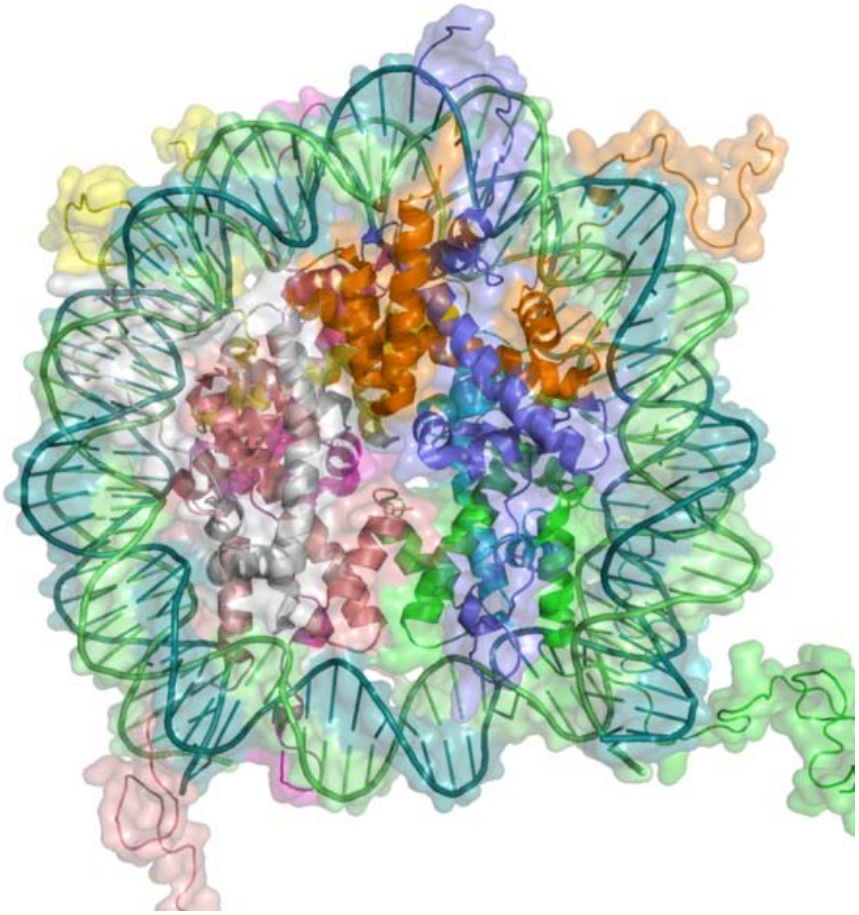
Direct interaction with DNA is the simplest and the most direct method by which a protein can change transcription levels. Genes often have several protein binding sites around the coding region with the specific function of regulating transcription. There are many classes of regulatory DNA binding sites known as enhancers, insulators, repressors and silencers. The mechanisms for regulating transcription are very varied, from blocking key binding sites on the DNA for RNA polymerase to acting as an activator and promoting transcription by assisting RNA polymerase binding.

The activity of transcription factors is further modulated by intracellular signals causing protein post-translational modification including phosphorylated, acetylated, or

glycosylated. These changes influence a transcription factor's ability to bind, directly or indirectly, to promoter DNA, to recruit RNA polymerase, or to favor elongation of a newly synthetized RNA molecule.

The nuclear membrane in eukaryotes allows further regulation of transcription factors by the duration of their presence in the nucleus which is regulated by reversible changes in their structure and by binding of other proteins. Environmental stimuli or endocrine signals may cause modification of regulatory proteins eliciting cascades of intracellular signals, which result in regulation of gene expression.

More recently it has become apparent that there is a huge influence of non-DNA-sequence specific effects on translation. These effects are referred to as epigenetic and involve the higher order structure of DNA, non-sequence specific DNA binding proteins and chemical modification of DNA. In general epigenetic effects alter the accessibility of DNA to proteins and so modulate transcription.



In eukaryotes, DNA is organized in form of nucleosomes. Note how the DNA (blue and green) is tightly wrapped around the protein core made of histone octamer (ribbon coils), restricting access to the DNA. From PDB 1KX5.

DNA methylation is a widespread mechanism for epigenetic influence on gene expression and is seen in bacteria and eukaryotes and has roles in heritable transcription silencing and transcription regulation. In eukaryotes the structure of chromatin, controlled by the histone code, regulates access to DNA with significant impacts on the expression of genes in euchromatin and heterochromatin areas.

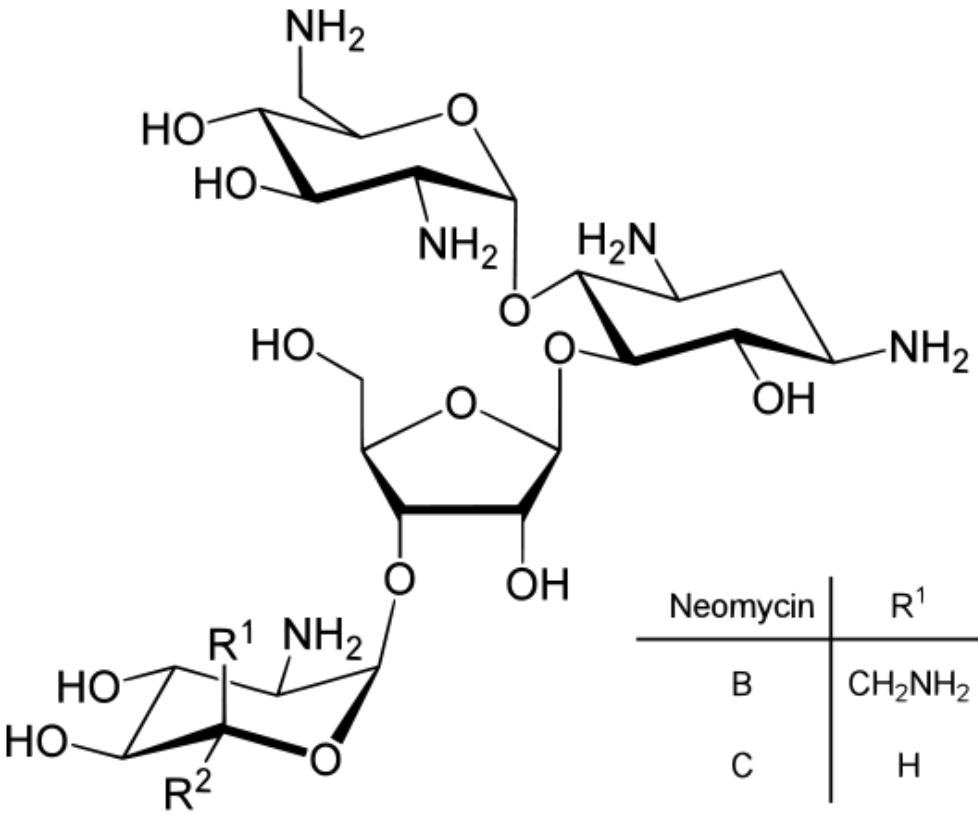
Post-transcriptional regulation

In eukaryotes, where export of RNA is required before translation is possible, nuclear export is thought to provide additional control over gene expression. All transport in and out of the nucleus is via the nuclear pore and transport is controlled by a wide range of importin and exportin proteins.

Expression of a gene coding for a protein is only possible if the messenger RNA carrying the code survives long enough to be translated. In a typical cell an RNA molecule is only stable if specifically protected from degradation. RNA degradation has particular importance in regulation of expression in eukaryotic cells where mRNA has to travel significant distances before being translated. In eukaryotes RNA is stabilised by certain post-transcriptional modifications, particularly the 5' cap and poly-adenylated tail.

Intentional degradation of mRNA is used not just as a defence mechanism from foreign RNA (normally from viruses) but also as a route of mRNA *destabilisation*. If an mRNA molecule has a complementary sequence to a small interfering RNA then it is targeted for destruction via the RNA interference pathway.

Translational regulation



Neomycin	R^1	R^2
B	CH_2NH_2	H
C	H	CH_2NH_2

Neomycin is an example of a small molecule which reduces expression of all protein genes inevitably leading to cell death, thus acts as an antibiotic.

Direct regulation of translation is less prevalent than control of transcription or mRNA stability but is occasionally used. Inhibition of protein translation is a major target for toxins and antibiotics in order to kill a cell by overriding its normal gene expression control. Protein synthesis inhibitors include the antibiotic neomycin and the toxin ricin.

Protein degradation

Once protein synthesis is complete the level of expression of that protein can be reduced by protein degradation. There are major protein degradation pathways in all prokaryotes and eukaryotes of which the proteasome is a common component. An unneeded or damaged protein is often labelled for degradation by addition of ubiquitin.

Measurement

Measuring gene expression is an important part of many life sciences - the ability to quantify the level at which a particular gene is expressed within a cell, tissue or organism can give a huge amount of information. For example measuring gene expression can:

- Identify viral infection of a cell (viral protein expression)
- Determine an individual's susceptibility to cancer (oncogene expression)
- Find if a bacterium is resistant to penicillin (beta-lactamase expression)

Similarly the analysis of the location of expression protein is a powerful tool and this can be done on an organism or cellular scale. Investigation of localisation is particularly important for study of development in multicellular organisms and as an indicator of protein function in single cells. Ideally measurement of expression is done by detecting the final gene product (for many genes this is the protein) however it is often easier to detect one of the precursors, typically mRNA, and infer gene expression level.

mRNA quantification

Levels of mRNA can be quantitatively measured by Northern blotting which gives size and sequence information about the mRNA molecules. A sample of RNA is separated on an agarose gel and hybridized to a radio-labeled RNA probe that is complementary to the target sequence. The radio-labeled RNA is then detected by an autoradiograph. The main problems with Northern blotting stem from the use of radioactive reagents (which make the procedure time consuming and potentially dangerous) and lower quality quantification than more modern methods (due to the fact that quantification is done by measuring band strength in an image of a gel). Northern blotting is, however, still widely used as the additional mRNA size information allows the discrimination of alternately spliced transcripts.

A more modern low-throughput approach for measuring mRNA abundance is reverse transcription quantitative polymerase chain reaction (RT-PCR followed with qPCR). RT-PCR first generates a DNA template from the mRNA by reverse transcription, which is called cDNA. This cDNA template is then used for qPCR where the change in fluorescence of a probe changes as the DNA amplification process progresses. With a carefully constructed standard curve qPCR can produce an absolute measurement such as number of copies of mRNA, typically in units of copies per nanolitre of homogenized tissue or copies per cell. qPCR is very sensitive (detection of a single mRNA molecule is possible), but can be expensive due to the fluorescent probes required.

Northern blots and RT-qPCR are good for detecting whether a single gene is being expressed, but it quickly becomes impractical if many genes within the sample are being studied. Using DNA microarrays transcript levels for many genes at once (expression profiling) can be measured. Recent advances in microarray technology allow for the quantification, on a single array, of transcript levels for every known gene in several organism's genomes, including humans.

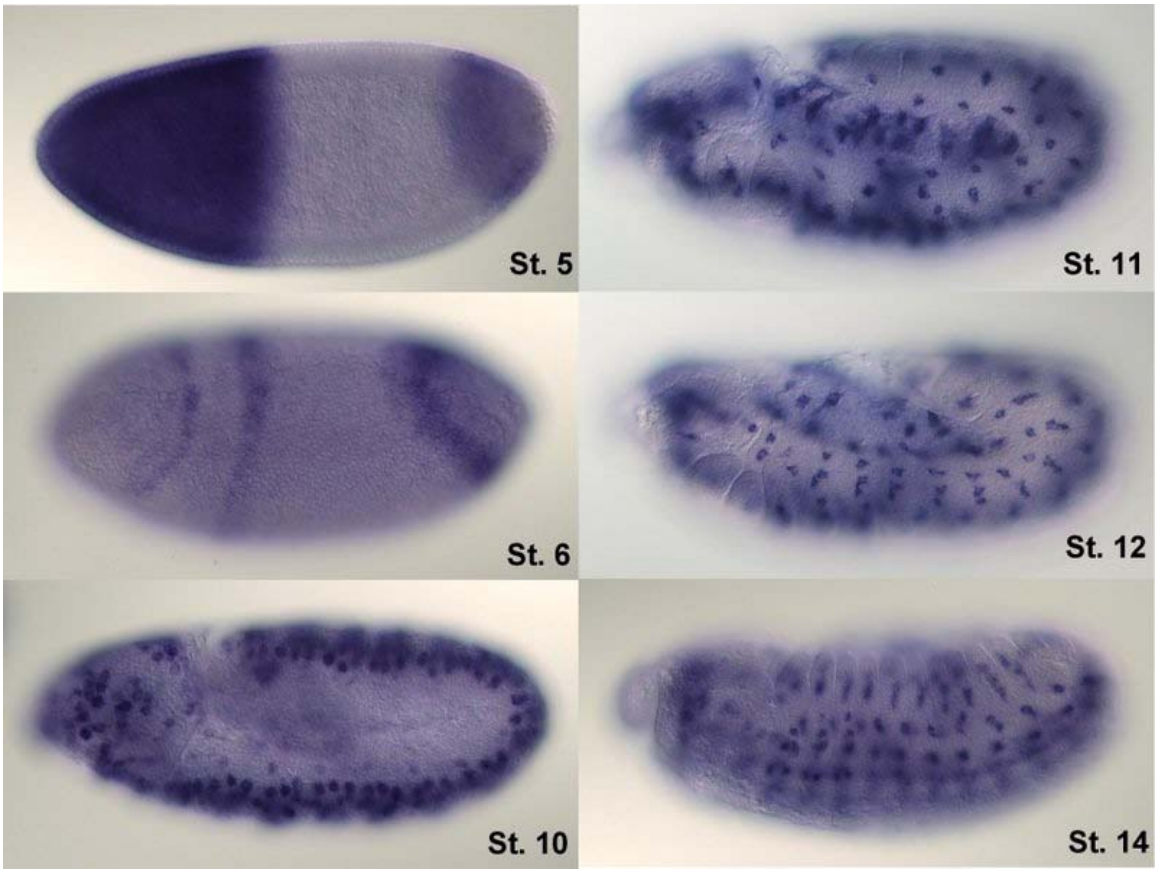
Alternatively "tag based" technologies like Serial analysis of gene expression (SAGE), which can provide a relative measure of the cellular concentration of different mRNAs, can be used. The great advantage of tag-based methods is the "open architecture", allowing for the exact measurement of any transcript, with a known or unknown sequence.

Protein quantification

For genes encoding proteins the expression level can be directly assessed by a number of means with some clear analogies to the techniques for mRNA quantification.

The most commonly used method is to perform a Western blot against the protein of interest - this gives information on the size of the protein in addition to its identity. A sample (often cellular lysate) is separated on a polyacrylamide gel, transferred to a membrane and then probed with an antibody to the protein of interest. The antibody can either be conjugated to a fluorophore or to horseradish peroxidase for imaging and/or quantification. The gel-based nature of this assay makes quantification less accurate but it has the advantage of being able to identify later modifications to the protein, for example proteolysis or ubiquitination, from changes in size.

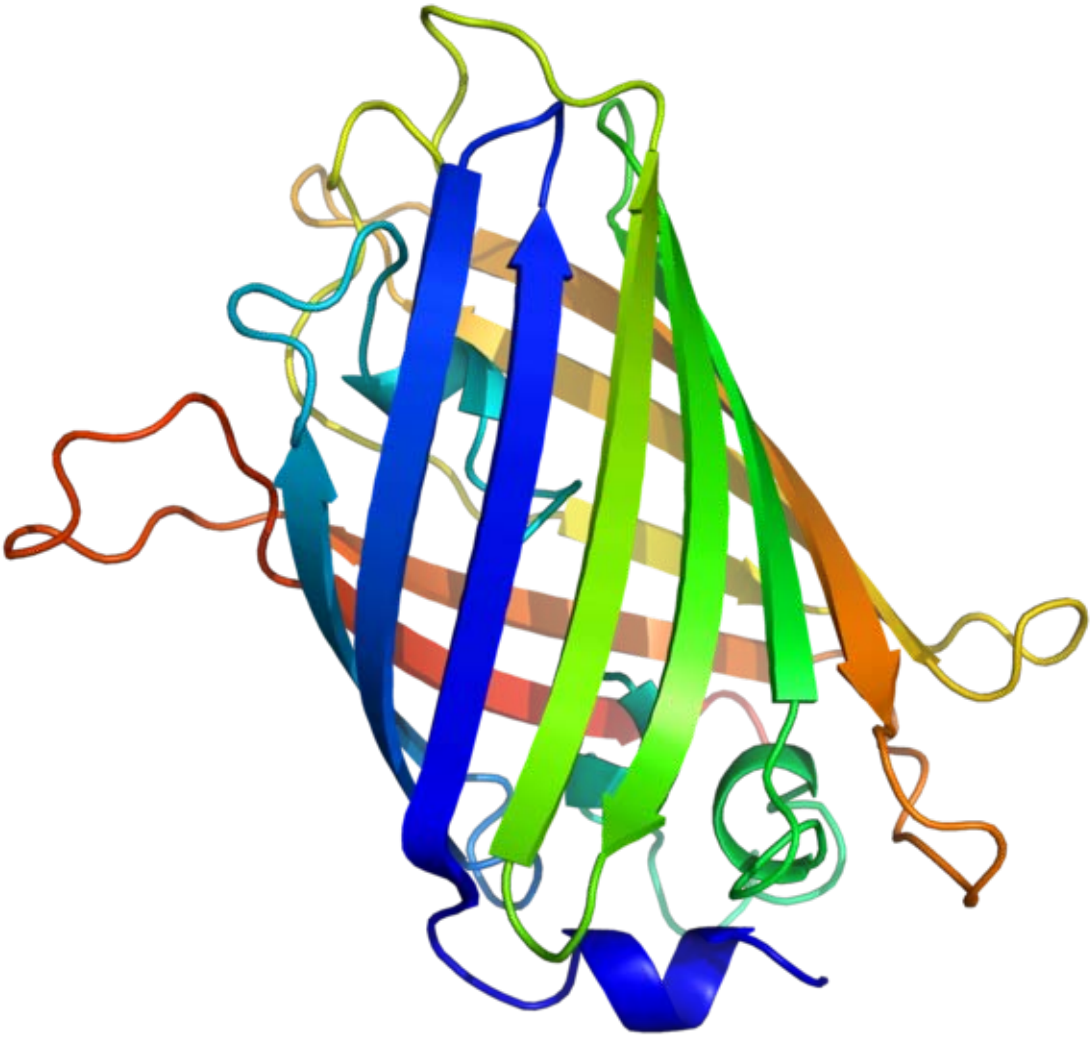
Localisation



In situ-hybridization of Drosophila embryos at different developmental stages for the mRNA responsible for the expression of hunchback. High intensity of blue color marks places with high hunchback mRNA quantity.

Analysis of expression is not limited to only quantification; localisation can also be determined. mRNA can be detected with a suitably labelled complementary mRNA

strand and protein can be detected via labelled antibodies. The probed sample is then observed by microscopy to identify where the mRNA or protein is.



The three-dimensional structure of green fluorescent protein. The residues in the centre of the "barrel" are responsible for production of green light after exposing to higher energetic blue light. From PDB 1EMA.

By replacing the gene with a new version fused a green fluorescent protein (or similar) marker expression may be directly quantified in live cells. This is done by imaging using a fluorescence microscope. It is very difficult to clone a GFP-fused protein into its native location in the genome without affecting expression levels so this method often cannot be used to measure endogenous gene expression. It is, however, widely used to measure the expression of a gene artificially introduced into the cell, for example via an expression vector. It is important to note that by fusing a target protein to a fluorescent reporter the

protein's behavior, including its cellular localization and expression level, can be significantly changed.

The enzyme-linked immunosorbent assay works by using antibodies immobilised on a microtiter plate to capture proteins of interest from samples added to the well. Using a detection antibody conjugated to an enzyme or fluorophore the quantity of bound protein can be accurately measured by fluorometric or colourimetric detection. The detection process is very similar to that of a Western blot, but by avoiding the gel steps more accurate quantification can be achieved.

Expression system

An expression system is a system specifically designed for the production of a gene product of choice. This is normally a protein although may also be RNA, such as tRNA or a ribozyme. An expression system consists of a gene, normally encoded by DNA, and the molecular machinery required to transcribe the DNA into mRNA and translate the mRNA into protein using the reagents provided. In the broadest sense this includes every living cell but the term is more normally used to refer to expression as a laboratory tool. An expression system is therefore often artificial in some manner. Expression systems are, however, a fundamentally natural process. Viruses are an excellent example where they replicate by using the host cell as an expression system for the viral proteins and genome.

In nature

In addition to these biological tools, certain naturally observed configurations of DNA (genes, promoters, enhancers, repressors) and the associated machinery itself are referred to as an expression system. This term is normally used in the case where a gene or set of genes is switched on under well defined conditions. For example the simple repressor switch expression system in Lambda phage and the lac operator system in bacteria. Several natural expression systems are directly used or modified and used for artificial expression systems such as the Tet-on and Tet-off expression system.

Gene networks

Genes have sometimes been regarded as nodes in a network, with inputs being proteins such as transcription factors, and outputs being the level of gene expression. The node itself performs a function, and the operation of these functions have been interpreted as performing a kind of information processing within cell and determine cellular behavior.

Gene networks can also be constructed without formulating an explicit causal model. This is often the case when assembling networks from large expression data sets. Covariation and correlation of expression is computed across a large sample of cases and measurements (often transcriptome or proteome data). The source of variation can be either experimental or natural (observational). There are several ways to construct gene

expression networks, but one common approach is to compute a matrix of all pair-wise correlations of expression across conditions, time points, or individuals and convert the matrix (after thresholding at some cut-off value) into a graphical representation in which nodes represent genes, transcripts, or proteins and edges connecting these nodes represent the strength of association.

Techniques and tools

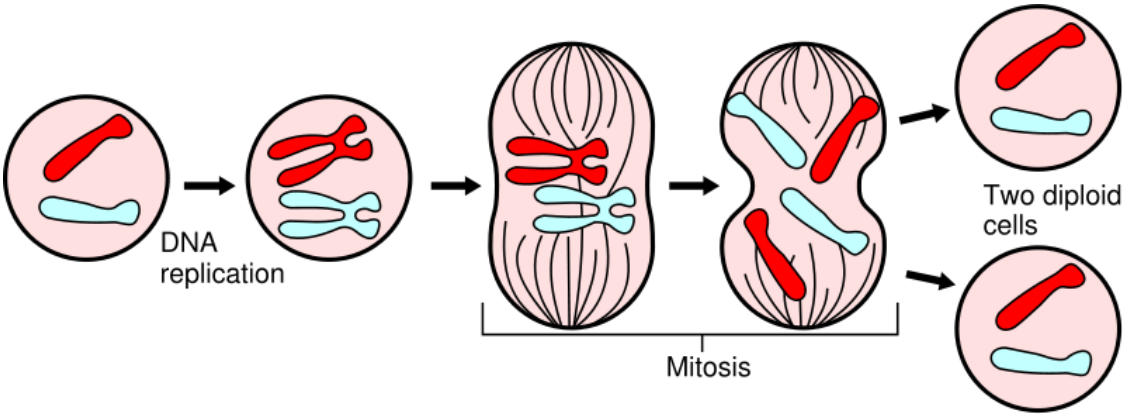
The following experimental techniques are used to measure gene expression and are listed in roughly chronological order, starting with the older, more established technologies. They are divided into two groups based on their degree of multiplexity.

- Low-to-mid-plex techniques:
  - Reporter gene
  - Northern blot
  - Western blot
  - Fluorescent in situ hybridization
  - Reverse transcription PCR

- Higher-plex techniques:
  - SAGE
  - DNA microarray
  - Tiling array
  - RNA-Seq

Chapter- 8

Cell Cycle

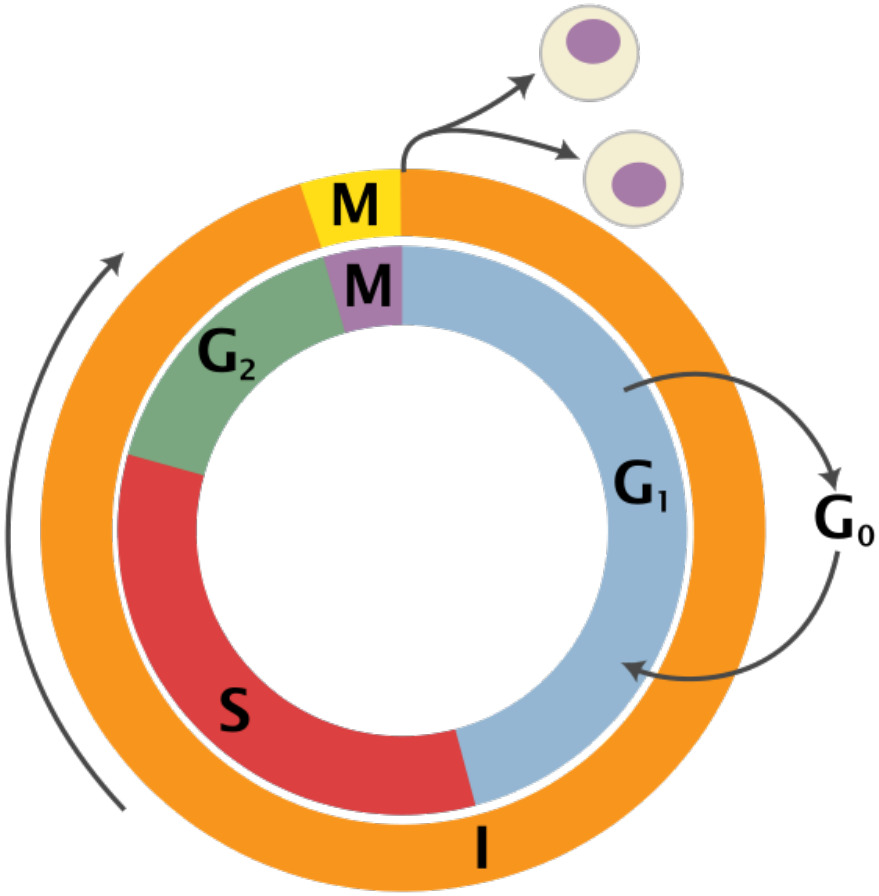


Each turn of the cell cycle divides the chromosomes in a cell nucleus.

The **cell cycle**, or **cell-division cycle**, is the series of events that takes place in a cell leading to its division and duplication (replication). In cells without a nucleus (prokaryotic), the cell cycle occurs via a process termed binary fission. In cells with a nucleus (eukaryotes), the cell cycle can be divided in two brief periods: interphase—during which the cell grows, accumulating nutrients needed for mitosis and duplicating its DNA—and the mitosis (M) phase, during which the cell splits itself into two distinct cells, often called "daughter cells". The cell-division cycle is a vital process by which a single-celled fertilized egg develops into a mature organism, as well as the process by which hair, skin, blood cells, and some internal organs are renewed.

Phases

The cell cycle consists of four distinct phases: G_1 phase, S phase (synthesis), G_2 phase (collectively known as interphase) and M phase (mitosis). M phase is itself composed of two tightly coupled processes: mitosis, in which the cell's chromosomes are divided between the two daughter cells, and cytokinesis, in which the cell's cytoplasm divides in half forming distinct cells. Activation of each phase is dependent on the proper progression and completion of the previous one. Cells that have temporarily or reversibly stopped dividing are said to have entered a state of quiescence called G_0 phase.



Schematic of the cell cycle. outer ring: I = Interphase, M = Mitosis; inner ring: M = Mitosis, G_1 = Gap 1, G_2 = Gap 2, S = Synthesis; not in ring: G_0 = Gap 0/Resting.

State	Phase	Abbreviation	Description
quiescent/ senescent	Gap 0	**G_0**	A resting phase where the cell has left the cycle and has stopped dividing.
Interphase	Gap 1	**G_1**	Cells increase in size in Gap 1. The *G_1 checkpoint* control mechanism ensures that everything is ready for DNA synthesis.
	Synthesis	**S**	DNA replication occurs during this phase.
	Gap 2	**G_2**	During the gap between DNA synthesis and mitosis, the cell will continue to grow. The *G_2 checkpoint* control mechanism ensures that everything is ready to enter the M (mitosis) phase and divide.

Cell division	Mitosis	**M**	Cell growth stops at this stage and cellular energy is focused on the orderly division into two daughter cells. A checkpoint in the middle of mitosis (*Metaphase Checkpoint*) ensures that the cell is ready to complete cell division.

After cell division, each of the daughter cells begin the interphase of a new cycle. Although the various stages of interphase are not usually morphologically distinguishable, each phase of the cell cycle has a distinct set of specialized biochemical processes that prepare the cell for initiation of cell division.

Resting (G_0 phase)

The term "post-mitotic" is sometimes used to refer to both quiescent and senescent cells. Nonproliferative cells in multicellular eukaryotes generally enter the quiescent G_0 state from G_1 and may remain quiescent for long periods of time, possibly indefinitely (as is often the case for neurons). This is very common for cells that are fully differentiated. Cellular senescence is a state that occurs in response to DNA damage or degradation that would make a cell's progeny nonviable; it is often a biochemical alternative to the self-destruction of such a damaged cell by apoptosis.

Interphase

Before a cell can enter cell division, it needs to take in nutrients. All of the preparations are done during the interphase. Interphase proceeds in three stages, G1, S, and G2. Cell division operates in a cycle. Therefore, interphase is preceded by the previous cycle of mitosis and cytokinesis.

G_1 phase

The first phase within interphase, from the end of the previous M phase until the beginning of DNA synthesis is called G_1 (G indicating *gap*). It is also called the growth phase. During this phase the biosynthetic activities of the cell, which had been considerably slowed down during M phase, resume at a high rate. This phase is marked by synthesis of various enzymes that are required in S phase, mainly those needed for DNA replication. Duration of G_1 is highly variable, even among different cells of the same species.

S phase

The ensuing S phase starts when DNA synthesis commences; when it is complete, all of the chromosomes have been replicated, i.e., each chromosome has two (sister) chromatids. Thus, during this phase, the amount of DNA in the cell has effectively doubled, though the ploidy of the cell remains the same. Rates of RNA transcription and

protein synthesis are very low during this phase. An exception to this is histone production, most of which occurs during the S phase.

G_2 phase

The cell then enters the G_2 phase, which lasts until the cell enters mitosis. Again, significant biosynthesis occurs during this phase, mainly involving the production of microtubules, which are required during the process of mitosis. Inhibition of protein synthesis during G_2 phase prevents the cell from undergoing mitosis.

Mitosis (M Phase/Mitotic phase)

The relatively brief M phase consists of nuclear division (karyokinesis). The M phase has been broken down into several distinct phases, sequentially known as:

- prophase,
- metaphase,
- anaphase,
- telophase
- cytokinesis (strictly speaking, cytokinesis is not part of mitosis but is an event that directly follows mitosis in which cytoplasm is divided into two daughter cells)

Mitosis is the process by which a eukaryotic cell separates the chromosomes in its cell nucleus into two identical sets in two nuclei. It is generally followed immediately by cytokinesis, which divides the nuclei, cytoplasm, organelles and cell membrane into two cells containing roughly equal shares of these cellular components. Mitosis and cytokinesis together define the **mitotic (M) phase** of the cell cycle - the division of the mother cell into two daughter cells, genetically identical to each other and to their parent cell. This accounts for approximately 10% of the cell cycle.

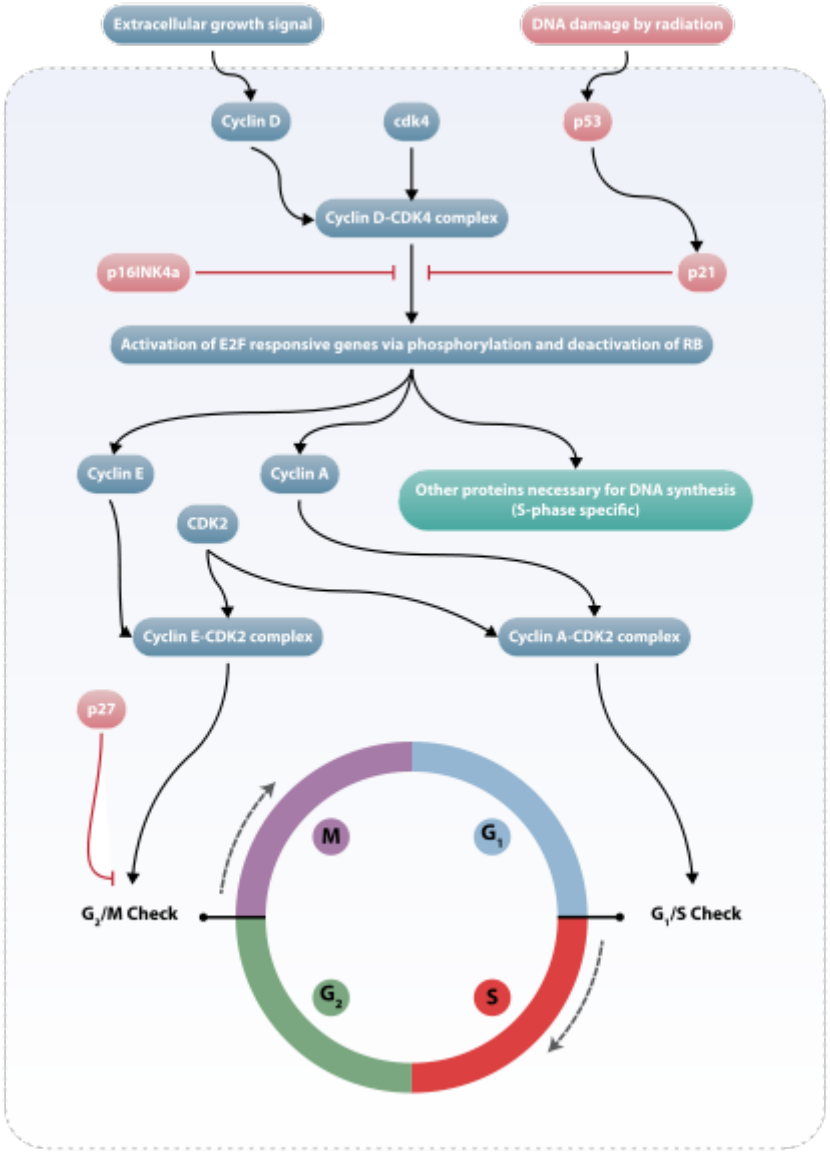
Mitosis occurs exclusively in eukaryotic cells, but occurs in different ways in different species. For example, animals undergo an "open" mitosis, where the nuclear envelope breaks down before the chromosomes separate, while fungi such as *Aspergillus nidulans* and *Saccharomyces cerevisiae* (yeast) undergo a "closed" mitosis, where chromosomes divide within an intact cell nucleus. Prokaryotic cells, which lack a nucleus, divide by a process called binary fission.

The process of mitosis is complex and highly regulated. The sequence of events is divided into phases, corresponding to the completion of one set of activities and the start of the next. These stages are prophase, prometaphase, metaphase, anaphase and telophase. During the process of mitosis the pairs of chromosomes condense and attach to fibers that pull the sister chromatids to opposite sides of the cell. The cell then divides in cytokinesis, to produce two identical daughter cells.

Because cytokinesis usually occurs in conjunction with mitosis, "mitosis" is often used interchangeably with "M phase". However, there are many cells where mitosis and

cytokinesis occur separately, forming single cells with multiple nuclei. This occurs most notably among the fungi and slime moulds, but is found in various different groups. Even in animals, cytokinesis and mitosis may occur independently, for instance during certain stages of fruit fly embryonic development. Errors in mitosis can either kill a cell through apoptosis or cause mutations that may lead to cancer.

Regulation of eukaryotic cell cycle



Regulation of cell cycle: Schematic

Regulation of the cell cycle involves processes crucial to the survival of a cell, including the detection and repair of genetic damage as well as the prevention of uncontrolled cell

division. The molecular events that control the cell cycle are ordered and directional; that is, each process occurs in a sequential fashion and it is impossible to "reverse" the cycle.

Role of cyclins and CDKs

Two key classes of regulatory molecules, cyclins and cyclin-dependent kinases (CDKs), determine a cell's progress through the cell cycle. Leland H. Hartwell, R. Timothy Hunt, and Paul M. Nurse won the 2001 Nobel Prize in Physiology or Medicine for their discovery of these central molecules. Many of the genes encoding cyclins and CDKs are conserved among all eukaryotes, but in general more complex organisms have more elaborate cell cycle control systems that incorporate more individual components. Many of the relevant genes were first identified by studying yeast, especially *Saccharomyces cerevisiae*; genetic nomenclature in yeast dubs many these genes *cdc* (for "cell division cycle") followed by an identifying number, e.g., *cdc25* or *cdc20*.

Cyclins form the regulatory subunits and CDKs the catalytic subunits of an activated heterodimer; cyclins have no catalytic activity and CDKs are inactive in the absence of a partner cyclin. When activated by a bound cyclin, CDKs perform a common biochemical reaction called phosphorylation that activates or inactivates target proteins to orchestrate coordinated entry into the next phase of the cell cycle. Different cyclin-CDK combinations determine the downstream proteins targeted. CDKs are constitutively expressed in cells whereas cyclins are synthesised at specific stages of the cell cycle, in response to various molecular signals.

General mechanism of cyclin-CDK interaction

Upon receiving a pro-mitotic extracellular signal, G_1 cyclin-CDK complexes become active to prepare the cell for S phase, promoting the expression of transcription factors that in turn promote the expression of S cyclins and of enzymes required for DNA replication. The G_1 cyclin-CDK complexes also promote the degradation of molecules that function as S phase inhibitors by targeting them for ubiquitination. Once a protein has been ubiquitinated, it is targeted for proteolytic degradation by the proteasome.

Active S cyclin-CDK complexes phosphorylate proteins that make up the pre-replication complexes assembled during G_1 phase on DNA replication origins. The phosphorylation serves two purposes: to activate each already-assembled pre-replication complex, and to prevent new complexes from forming. This ensures that every portion of the cell's genome will be replicated once and only once. The reason for prevention of gaps in replication is fairly clear, because daughter cells that are missing all or part of crucial genes will die. However, for reasons related to gene copy number effects, possession of extra copies of certain genes is also deleterious to the daughter cells.

Mitotic cyclin-CDK complexes, which are synthesized but inactivated during S and G_2 phases, promote the initiation of mitosis by stimulating downstream proteins involved in chromosome condensation and mitotic spindle assembly. A critical complex activated during this process is a ubiquitin ligase known as the anaphase-promoting complex

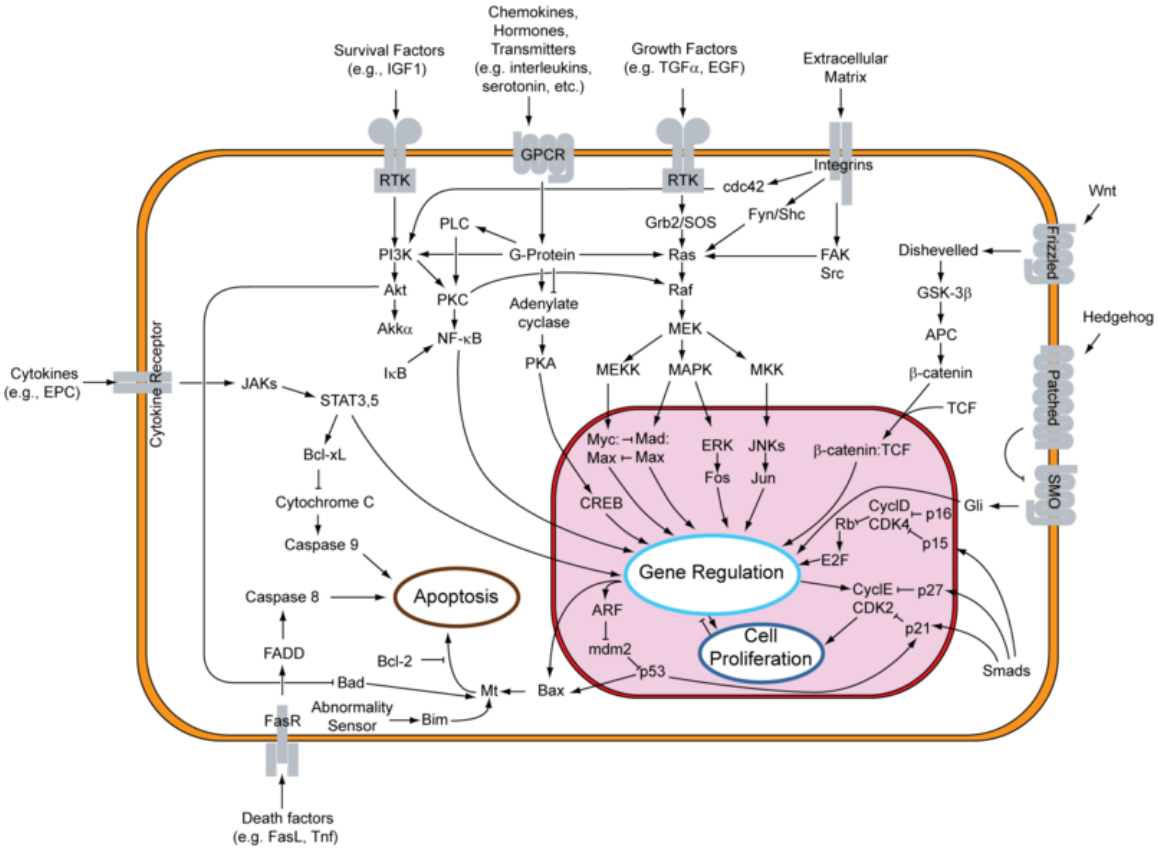
(APC), which promotes degradation of structural proteins associated with the chromosomal kinetochore. APC also targets the mitotic cyclins for degradation, ensuring that telophase and cytokinesis can proceed.

Interphase: Interphase generally lasts at least 12 to 24 hours in mammalian tissue. During this period, the cell is constantly synthesizing RNA, producing protein and growing in size. By studying molecular events in cells, scientists have determined that interphase can be divided into 4 steps: Gap 0 (G_0), Gap 1 (G_1), S (synthesis) phase, Gap 2 (G_2).

Specific action of cyclin-CDK complexes

Cyclin D is the first cyclin produced in the cell cycle, in response to extracellular signals (e.g. growth factors). Cyclin D binds to existing CDK4, forming the active cyclin D-CDK4 complex. Cyclin D-CDK4 complex in turn phosphorylates the retinoblastoma susceptibility protein (Rb). The hyperphosphorylated Rb dissociates from the E2F/DP1/Rb complex (which was bound to the E2F responsive genes, effectively "blocking" them from transcription), activating E2F. Activation of E2F results in transcription of various genes like cyclin E, cyclin A, DNA polymerase, thymidine kinase, etc. Cyclin E thus produced binds to CDK2, forming the cyclin E-CDK2 complex, which pushes the cell from G_1 to S phase (G_1/S transition). Cyclin B along with cdc2 (cdc2 - fission yeasts (CDK1 - mammalia)) forms the cyclin B-cdc2 complex, which initiates the G_2/M transition. Cyclin B-cdc2 complex activation causes breakdown of nuclear envelope and initiation of prophase, and subsequently, its deactivation causes the cell to exit mitosis.

Inhibitors



Overview of signal transduction pathways involved in apoptosis, also known as "programmed cell death".

Two families of genes, the *cip/kip* family and the INK4a/ARF (*In*hibitor of *K*inase 4/*A*lternative *R*eading *F*rame) prevent the progression of the cell cycle. Because these genes are instrumental in prevention of tumor formation, they are known as tumor suppressors.

The ***cip/kip* family** includes the genes p21, p27 and p57. They halt cell cycle in G_1 phase, by binding to, and inactivating, cyclin-CDK complexes. p21 is activated by p53 (which, in turn, is triggered by DNA damage e.g. due to radiation). p27 is activated by Transforming Growth Factor β (TGF β), a growth inhibitor.

The **INK4a/ARF family** includes p16INK4a, which binds to CDK4 and arrests the cell cycle in G_1 phase, and p14arf which prevents p53 degradation.

Synthetic inhibitors of Cdc25 could also be useful for the arrest of cell cycle and therefore be useful as antineoplastic and anticancer agents.

Transcriptional Regulatory Network

Evidence suggests that a semi-autonomous transcriptional network acts in concert with the CDK-cyclin machinery to regulate the cell cycle. Several gene expression studies in *Saccharomyces cerevisiae* have identified approximately 800 to 1200 genes that change expression over the course of the cell cycle; they are transcribed at high levels at specific points in the cell cycle, and remain at lower levels throughout the rest of the cell cycle. While the set of identified genes differs between studies due to the computational methods and criterion used to identify them, each study indicates that a large portion of yeast genes are temporally regulated.

Many periodically expressed genes are driven by transcription factors that are also periodically expressed. One screen of single-gene knockouts identified 48 transcription factors (about 20% of all non-essential transcription factors) that show cell cycle progression defects. Genome-wide studies using high throughput technologies have identified the transcription factors that bind to the promoters of yeast genes, and correlating these findings with temporal expression patterns have allowed the identification of transcription factors that drive phase-specific gene expression. The expression profiles of these transcription factors are driven by the transcription factors that peak in the prior phase, and computational models have shown that a CDK-autonomous network of these transcription factors is sufficient to produce steady-state oscillations in gene expression).

Experimental evidence also suggests that gene expression can oscillate with the period seen in dividing wild-type cells independently of the CDK machinery. Orlando et. al. used microarrays to measure the expression of a set of 1,271 genes that they identified as periodic in both wild type cells and cells lacking all S-phase and mitotic cyclins (*clb1,2,3,4,5,6*). Of the 1,271 genes assayed, 882 continued to be expressed in the cyclin-deficient cells at the same time as in the wild type cells, despite the fact that the cyclin-deficient cells arrest at the border between G1 and S phase. However, 833 of the genes assayed changed behavior between the wild type and mutant cells, indicating that these genes are likely directly or indirectly regulated by the CDK-cyclin machinery. Some genes that continued to be expressed on time in the mutant cells were also expressed at different levels in the mutant and wild type cells. These findings suggest that while the transcriptional network may oscillate independently of the CDK-cyclin oscillator, they are coupled in a manner that requires both to ensure the proper timing of cell cycle events. Other work indicates that phosphorylation, a post-translational modification, of cell cycle transcription factors by Cdk1 may alter the localization or activity of the transcription factors in order to tightly control timing of target genes (Ubersax et al 2003; Sidorova et al 1995; White et al 2009).

While oscillatory transcription plays a key role in the progression of the yeast cell cycle, the CDK-cyclin machinery operates independently in the early embryonic cell cycle. Before the midblastula transition, zygotic transcription does not occur and all needed proteins, such as the B-type cyclins, are translated from maternally loaded mRNA.

Checkpoints

Cell cycle checkpoints are used by the cell to monitor and regulate the progress of the cell cycle. Checkpoints prevent cell cycle progression at specific points, allowing verification of necessary phase processes and repair of DNA damage. The cell cannot proceed to the next phase until checkpoint requirements have been met.

Several checkpoints are designed to ensure that damaged or incomplete DNA is not passed on to daughter cells. Two main checkpoints exist: the G_1/S checkpoint and the G_2/M checkpoint. G_1/S transition is a rate-limiting step in the cell cycle and is also known as restriction point. An alternative model of the cell cycle response to DNA damage has also been proposed, known as the postreplication checkpoint.

p53 plays an important role in triggering the control mechanisms at both G_1/S and G_2/M checkpoints.

Role in tumor formation

A disregulation of the cell cycle components may lead to tumor formation. As mentioned above, some genes like the cell cycle inhibitors, RB, p53 etc., when they mutate, may cause the cell to multiply uncontrollably, forming a tumor. Although the duration of cell cycle in tumor cells is equal to or longer than that of normal cell cycle, the proportion of cells that are in active cell division (versus quiescent cells in G_0 phase) in tumors is much higher than that in normal tissue. Thus there is a net increase in cell number as the number of cells that die by apoptosis or senescence remains the same.

The cells which are actively undergoing cell cycle are targeted in cancer therapy as the DNA is relatively exposed during cell division and hence susceptible to damage by drugs or radiation. This fact is made use of in cancer treatment; by a process known as debulking, a significant mass of the tumor is removed which pushes a significant number of the remaining tumor cells from G_0 to G_1 phase (due to increased availability of nutrients, oxygen, growth factors etc.). Radiation or chemotherapy following the debulking procedure kills these cells which have newly entered the cell cycle.

The fastest cycling mammalian cells in culture, crypt cells in the intestinal epithelium, have a cycle time as short as 9 to 10 hours. Stem cells in resting mouse skin may have a cycle time of more than 200 hours. Most of this difference is due to the varying length of G_1, the most variable phase of the cycle. M and S do not vary much.

In general, cells are most radiosensitive in late M and G_2 phases and most resistant in late S.

For cells with a longer cell cycle time and a significantly long G_1 phase, there is a second peak of resistance late in G_1

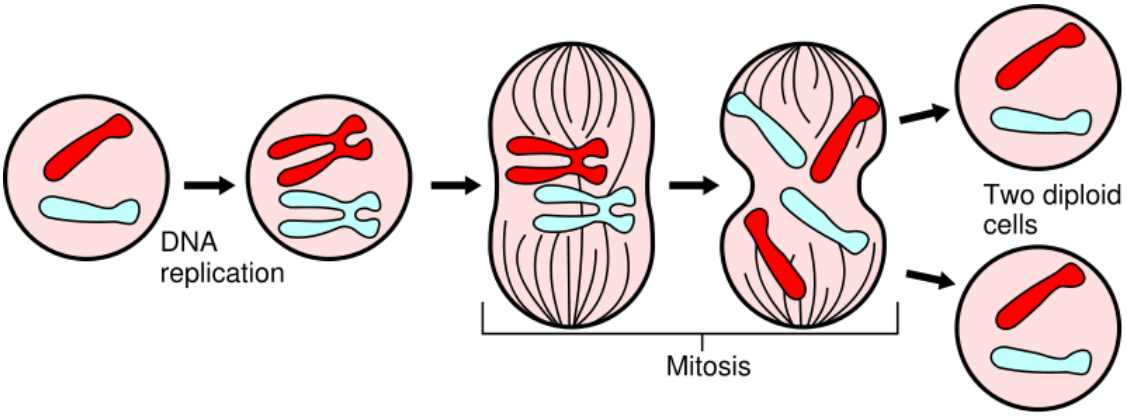
The pattern of resistance and sensitivity correlates with the level of sulfhydryl compounds in the cell. Sulfhydryls are natural radioprotectors and tend to be at their highest levels in S and at their lowest near mitosis.

Synchronization of cell cultures

Several methods can be used to synchronise cell cultures by halting the cell cycle at a particular phase. For example, serum starvation and treatment with thymidine or aphidicolin halt the cell in the G_1 phase, mitotic shake-off, treatment with colchicine and treatment with nocodazole halt the cell in M phase and treatment with 5-fluorodeoxyuridine halts the cell in S phase.

Chapter- 9

Mitosis



Mitosis divides the chromosomes in a cell nucleus.

Mitosis is the process by which a eukaryotic cell separates the chromosomes in its cell nucleus into two identical sets in two nuclei. It is generally followed immediately by cytokinesis, which divides the nuclei, cytoplasm, organelles and cell membrane into two cells containing roughly equal shares of these cellular components. Mitosis and cytokinesis together define the **mitotic (M) phase** of the cell cycle—the division of the mother cell into two daughter cells, genetically identical to each other and to their parent cell. This accounts for approximately 10% of the cell cycle.

Mitosis occurs exclusively in eukaryotic cells, but the process varies in different species. For example, animals undergo an "open" mitosis, where the nuclear envelope breaks down before the chromosomes separate, while fungi such as *Aspergillus nidulans* and *Saccharomyces cerevisiae* (yeast) undergo a "closed" mitosis, where chromosomes divide within an intact cell nucleus. Prokaryotic cells, which lack a nucleus, divide by a process called binary fission.

The process of mitosis is complex and highly regulated. The sequence of events is divided into phases, corresponding to the completion of one set of activities and the start of the next. These stages are interphase, prophase, prometaphase, metaphase, anaphase and telophase. During mitosis the pairs of chromosomes condense and attach to fibers

that pull the sister chromatids to opposite sides of the cell. The cell then divides in cytokinesis, to produce two identical daughter cells.

Because cytokinesis usually occurs in conjunction with mitosis, "mitosis" is often used interchangeably with "mitotic phase". However, there are many cells where mitosis and cytokinesis occur separately, forming single cells with multiple nuclei. This occurs most notably among the fungi and slime moulds, but is found in various different groups. Even in animals, cytokinesis and mitosis may occur independently, for instance during certain stages of fruit fly embryonic development. Errors in mitosis can either kill a cell through apoptosis or cause mutations that may lead to cancer.

Overview

The primary result of mitosis is the transferring of the parent cell's genome into two daughter cells. The genome is composed of a number of chromosomes—complexes of tightly-coiled DNA that contain genetic information vital for proper cell function. Because each resultant daughter cell should be genetically identical to the parent cell, the parent cell must make a copy of each chromosome before mitosis. This occurs during the S phase of interphase, the period that precedes the mitotic phase in the cell cycle where preparation for mitosis occurs.

Each new chromosome now contains two identical copies of itself, called *sister chromatids*, attached together in a specialized region of the chromosome known as the *centromere*. Each sister chromatid is not considered a chromosome in itself, and a chromosome always contains two sister chromatids.

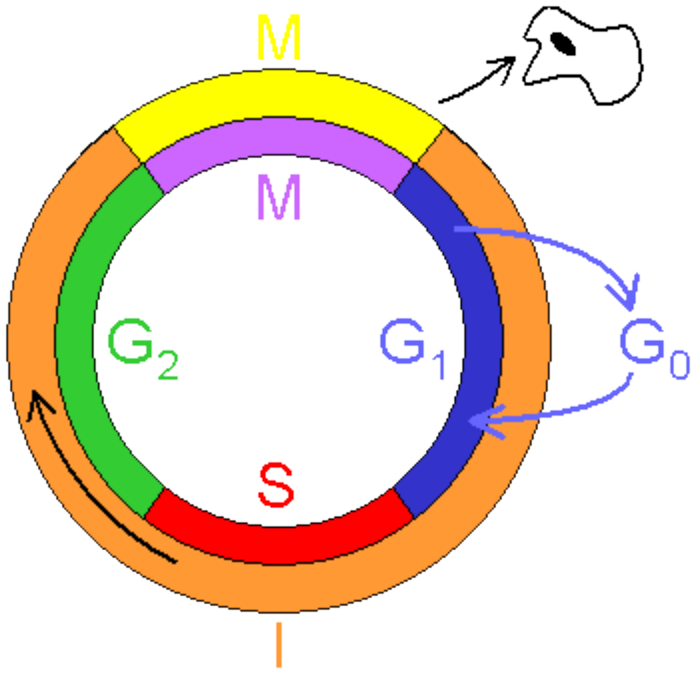
In most eukaryotes, the nuclear envelope that combines the DNA from the cytoplasm disassembles. The chromosomes align themselves in a line spanning the cell. Microtubules, essentially miniature strings, splay out from opposite ends of the cell and shorten, pulling apart the sister chromatids of each chromosome. As a matter of convention, each sister chromatid is now considered a chromosome, so they are renamed to *sister chromosomes*. As the cell elongates, corresponding sister chromosomes are pulled toward opposite ends. A new nuclear envelope forms around the separated sister chromosomes.

As mitosis completes cytokinesis is well underway. In animal cells, the cell pinches inward where the imaginary line used to be (the area of the cell membrane that pinches to form the two daughter cells is called the cleavage furrow), separating the two developing nuclei. In plant cells, the daughter cells will construct a new dividing cell wall between each other. Eventually, the mother cell will be split in half, giving rise to two daughter cells, each with an equivalent and complete copy of the original genome.

Prokaryotic cells undergo a process similar to mitosis called binary fission. However, prokaryotes cannot be properly said to undergo cytokinesis because they lack a nucleus and only have a single chromosome with no mitochondria.

Phases of cell cycle and mitosis

Interphase



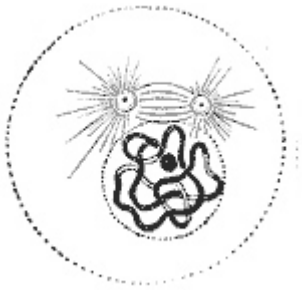
The cell cycle

The mitotic phase is a relatively short period of the cell cycle. It alternates with the much longer *interphase*, where the cell prepares itself for cell division. Interphase is therefore not part of mitosis. Interphase is divided into three phases, G_1 (first gap), S (synthesis), and G_2 (second gap). During all three phases, the cell grows by producing proteins and cytoplasmic organelles. However, chromosomes are replicated only during the S phase. Thus, a cell grows (G_1), continues to grow as it duplicates its chromosomes (S), grows more and prepares for mitosis (G_2), and finally it divides (M) before restarting the cycle.

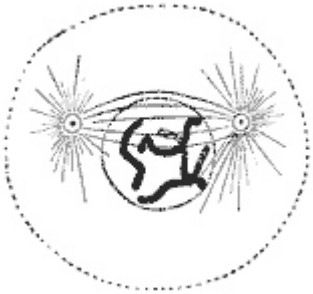
Preprophase

In plant cells only, prophase is preceded by a pre-prophase stage. In highly vacuolated plant cells, the nucleus has to migrate into the center of the cell before mitosis can begin. This is achieved through the formation of a phragmosome, a transverse sheet of cytoplasm that bisects the cell along the future plane of cell division. In addition to phragmosome formation, preprophase is characterized by the formation of a ring of microtubules and actin filaments (called preprophase band) underneath the plasma membrane around the equatorial plane of the future mitotic spindle. This band marks the position where the cell will eventually divide. The cells of higher plants (such as the flowering plants) lack centrioles; instead, microtubules form a spindle on the surface of the nucleus and are then being organized into a spindle by the chromosomes themselves,

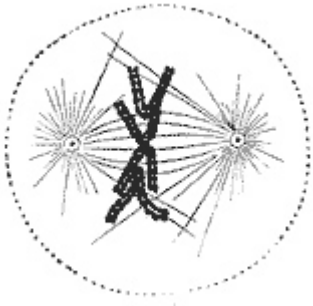
after the nuclear membrane breaks down. The preprophase band disappears during nuclear envelope disassembly and spindle formation in prometaphase.



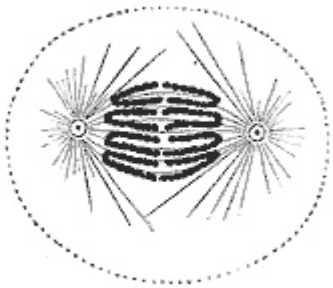
Prophase: The two round objects above the nucleus are the centrosomes. The chromatin has condensed.



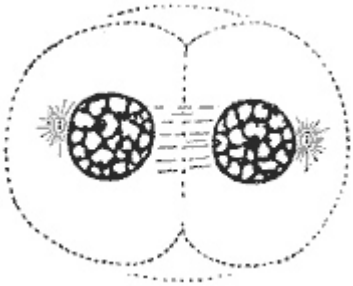
Prometaphase: The nuclear membrane has degraded, and microtubules have invaded the nuclear space. These microtubules can attach to kinetochores or they can interact with opposing microtubules.



Metaphase: The chromosomes have aligned at the metaphase plate.

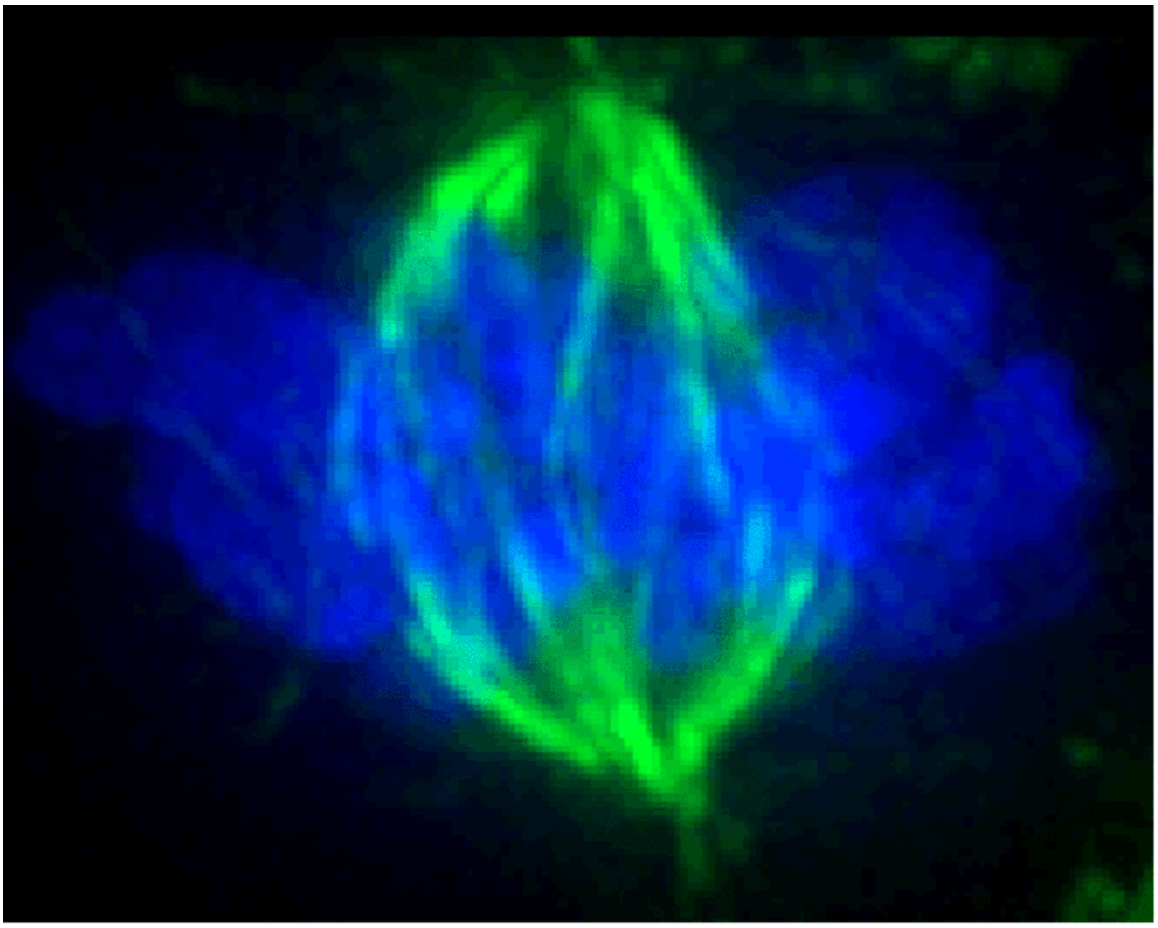


Early anaphase: The kinetochore microtubules shorten.



Telophase: The decondensing chromosomes are surrounded by nuclear membranes. Cytokinesis has already begun; the pinched area is known as the *cleavage furrow*.

Prophase



Micrograph showing condensed chromosomes in blue and the mitotic spindle in green during prometaphase of mitosis

Normally, the genetic material in the nucleus is in a loosely bundled coil called chromatin. At the onset of prophase, chromatin condenses together into a highly ordered structure called a chromosome. Since the genetic material has already been duplicated earlier in S phase, the replicated chromosomes have two sister chromatids, bound together at the centromere by the cohesion complex. Chromosomes are typically visible at high magnification through a light microscope.

Close to the nucleus are structures called centrosomes, which are made of a pair of centrioles. The centrosome is the coordinating center for the cell's microtubules. A cell inherits a single centrosome at cell division, which replicates before a new mitosis begins, giving a pair of centrosomes. The two centrosomes nucleate microtubules (which may be thought of as cellular ropes or poles) to form the spindle by polymerizing soluble tubulin. Molecular motor proteins then push the centrosomes along these microtubules to opposite sides of the cell. Although centrioles help organize microtubule assembly, they are not essential for the formation of the spindle, since they are absent from plants, and centrosomes are not always used in meiosis.

Prometaphase

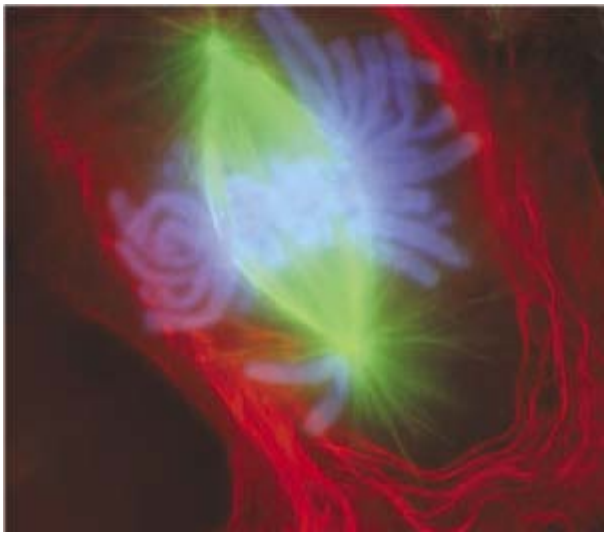
The nuclear envelope disassembles and microtubules invade the nuclear space. This is called *open mitosis*, and it occurs in most multicellular organisms. Fungi and some protists, such as algae or trichomonads, undergo a variation called *closed mitosis* where the spindle forms inside the nucleus, or its microtubules are able to penetrate an intact nuclear envelope.

Each chromosome forms two kinetochores at the centromere, one attached at each chromatid. A kinetochore is a complex protein structure that is analogous to a ring for the microtubule hook; it is the point where microtubules attach themselves to the chromosome. Although the kinetochore structure and function are not fully understood, it is known that it contains some form of molecular motor. When a microtubule connects with the kinetochore, the motor activates, using energy from ATP to "crawl" up the tube toward the originating centrosome. This motor activity, coupled with polymerisation and depolymerisation of microtubules, provides the pulling force necessary to later separate the chromosome's two chromatids.

When the spindle grows to sufficient length, *kinetochore microtubules* begin searching for kinetochores to attach to. A number of *nonkinetochore microtubules* find and interact with corresponding nonkinetochore microtubules from the opposite centrosome to form the mitotic spindle. Prometaphase is sometimes considered part of prophase.

In the fishing pole analogy, the kinetochore would be the "hook" that catches a sister chromatid or "fish". The centrosome acts as the "reel" that draws in the spindle fibers or "fishing line".

Metaphase



A cell in late metaphase. All chromosomes (blue) but one have arrived at the metaphase plate.

As microtubules find and attach to kinetochores in prometaphase, the centromeres of the chromosomes convene along the *metaphase plate* or *equatorial plane*, an imaginary line that is equidistant from the two centrosome poles. This even alignment is due to the counterbalance of the pulling powers generated by the opposing kinetochores, analogous to a tug-of-war between people of equal strength. In certain types of cells, chromosomes do not line up at the metaphase plate and instead move back and forth between the poles randomly, only roughly lining up along the midline. Metaphase comes from the Greek *μετα* meaning "after."

Because proper chromosome separation requires that every kinetochore be attached to a bundle of microtubules (spindle fibres), it is thought that unattached kinetochores generate a signal to prevent premature progression to anaphase without all chromosomes being aligned. The signal creates the *mitotic spindle checkpoint*.

Anaphase

When every kinetochore is attached to a cluster of microtubules and the chromosomes have lined up along the metaphase plate, the cell proceeds to anaphase (from the Greek *ανα* meaning “up,” “against,” “back,” or “re-”).

Two events then occur: first, the proteins that bind sister chromatids together are cleaved, allowing them to separate. These sister chromatids, which have now become distinct sister chromosomes, are pulled apart by shortening kinetochore microtubules and move toward the respective centrosomes to which they are attached. Next, the nonkinetochore microtubules elongate, pulling the centrosomes (and the set of chromosomes to which they are attached) apart to opposite ends of the cell. The force that causes the centrosomes to move towards the ends of the cell is still unknown, although there is a theory that suggests that the rapid assembly and breakdown of microtubules may cause this movement.

These two stages are sometimes called early and late anaphase. Early anaphase is usually defined as the separation of the sister chromatids, while late anaphase is the elongation of the microtubules and the chromosomes being pulled farther apart. At the end of anaphase, the cell has succeeded in separating identical copies of the genetic material into two distinct populations.

Telophase

Telophase (from the Greek *τελος* meaning "end") is a reversal of prophase and prometaphase events. It "cleans up" the after effects of mitosis. At telophase, the nonkinetochore microtubules continue to lengthen, elongating the cell even more. Corresponding sister chromosomes attach at opposite ends of the cell. A new nuclear envelope, using fragments of the parent cell's nuclear membrane, forms around each set of separated sister chromosomes. Both sets of chromosomes, now surrounded by new nuclei, unfold back into chromatin. Mitosis is complete, but cell division is not yet complete.

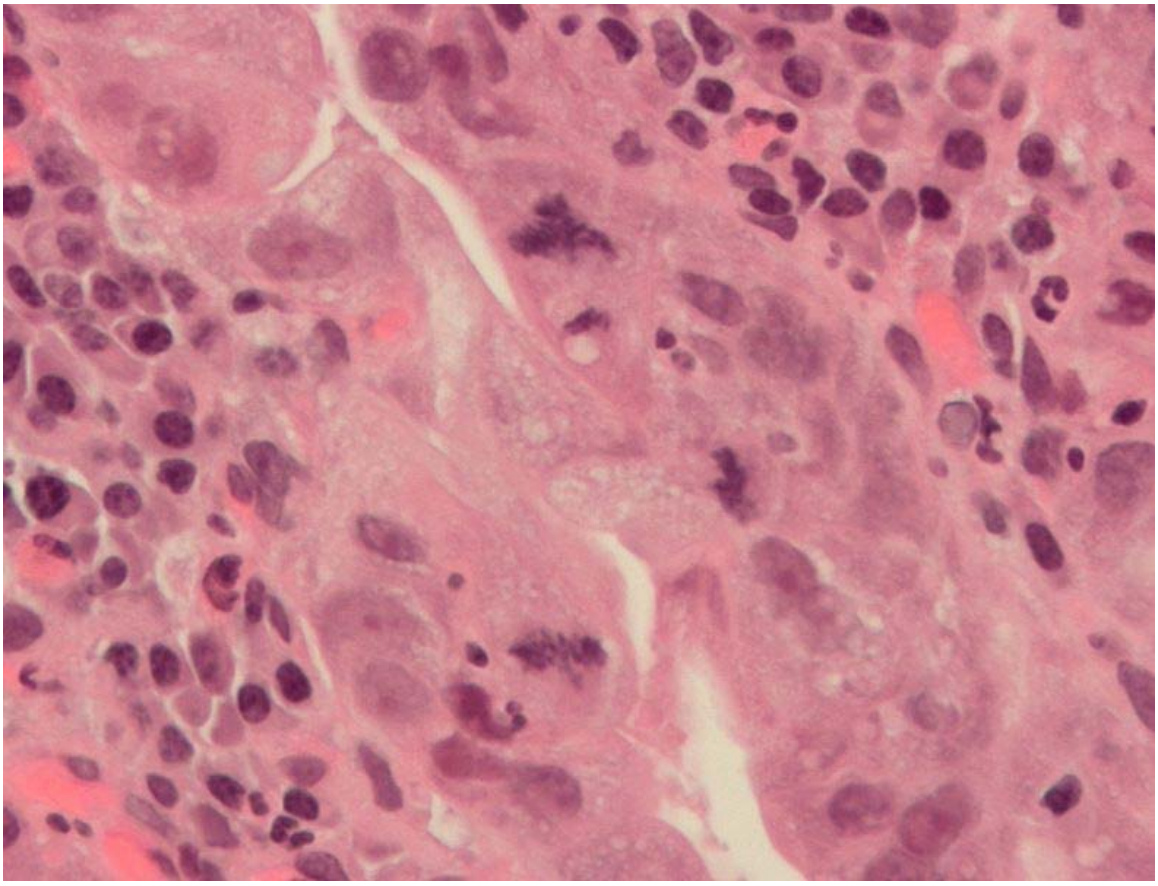
Cytokinesis

Cytokinesis is often mistakenly thought to be the final part of telophase; however, cytokinesis is a separate process that begins at the same time as telophase. Cytokinesis is technically not even a phase of mitosis, but rather a separate process, necessary for completing cell division. In animal cells, a cleavage furrow (pinch) containing a contractile ring develops where the metaphase plate used to be, pinching off the separated nuclei. In both animal and plant cells, cell division is also driven by vesicles derived from the Golgi apparatus, which move along microtubules to the middle of the cell. In plants this structure coalesces into a cell plate at the center of the phragmoplast and develops into a cell wall, separating the two nuclei. The phragmoplast is a microtubule structure typical for higher plants, whereas some green algae use a phycoplast microtubule array during cytokinesis. Each daughter cell has a complete copy of the genome of its parent cell. The end of cytokinesis marks the end of the M-phase.

Significance

Mitosis is important for the maintenance of the chromosomal set; each cell formed receives chromosomes that are alike in composition and equal in number to the chromosomes of the parent cell. Transcription is generally believed to cease during mitosis, but epigenetic mechanisms such as bookmarking function during this stage of the cell cycle to ensure that the "memory" of which genes were active prior to entry into mitosis are transmitted to the daughter cells.

Consequences of errors



An abnormal (tripolar) mitoses (12 o'clock position) in a precancerous lesion of the stomach. H&E stain

Although errors in mitosis are rare, the process may go wrong, especially during early cellular divisions in the zygote. Mitotic errors can be especially dangerous to the organism because future offspring from this parent cell will carry the same disorder.

In *non-disjunction*, a chromosome may fail to separate during anaphase. One daughter cell will receive both sister chromosomes and the other will receive none. This results in the former cell having three chromosomes containing the same genes (two sisters and a homologue), a condition known as *trisomy*, and the latter cell having only one chromosome (the homologous chromosome), a condition known as *monosomy*. These cells are considered aneuploid, a condition often associated with cancer.

Mitosis is a demanding process for the cell, which goes through dramatic changes in ultrastructure, its organelles disintegrate and reform in a matter of hours, and chromosomes are jostled constantly by probing microtubules. Occasionally, chromosomes may become damaged. An arm of the chromosome may be broken and the fragment lost, causing deletion. The fragment may incorrectly reattach to another, non-

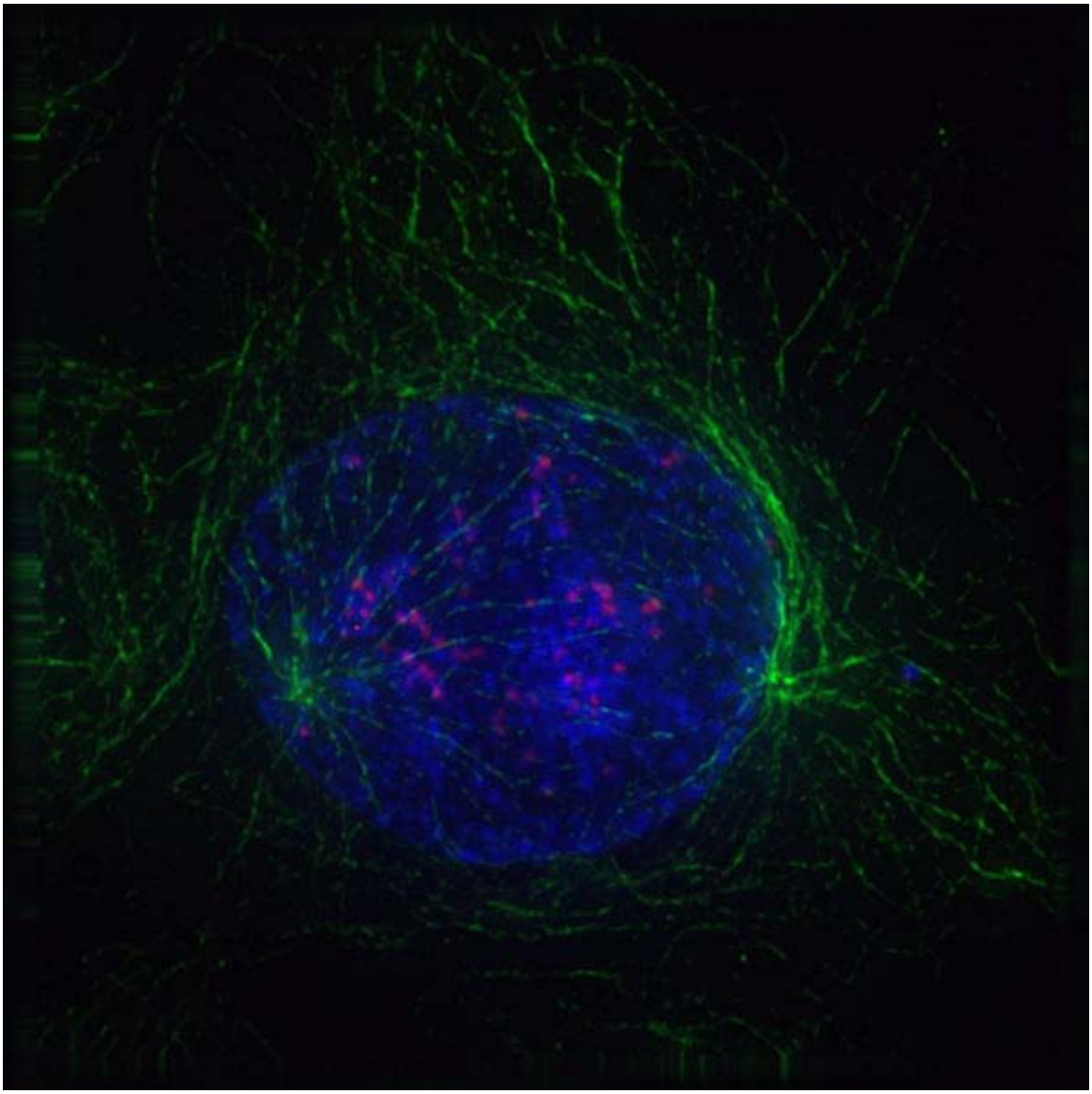
homologous chromosome, causing translocation. It may reattach to the original chromosome, but in reverse orientation, causing inversion. Or, it may be treated erroneously as a separate chromosome, causing chromosomal duplication. The effect of these genetic abnormalities depends on the specific nature of the error. It sometimes ranges from no noticeable effect to cancer induction or organism death.

Endomitosis

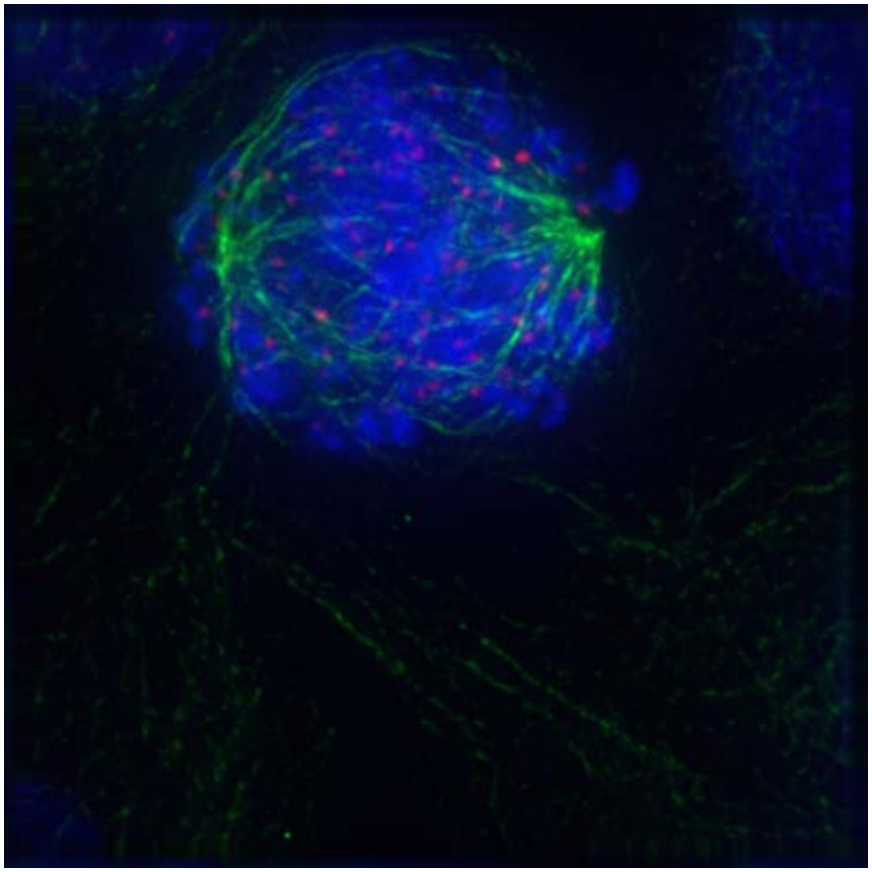
Endomitosis is a variant of mitosis without nuclear or cellular division, resulting in cells with many copies of the same chromosome occupying a single nucleus. This process may also be referred to as endoreduplication and the cells as endoploid. An example of a cell that goes through endomitosis is the megakaryocyte.

Timeline in pictures

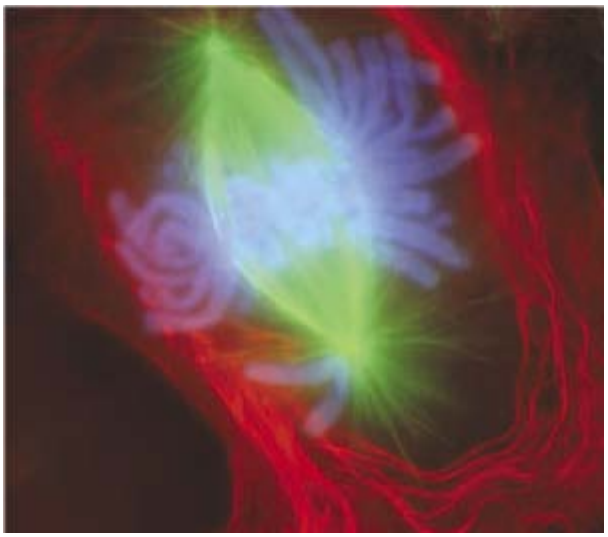
Real mitotic cells can be visualized through the microscope by staining them with fluorescent antibodies and dyes. These light micrographs are included below.



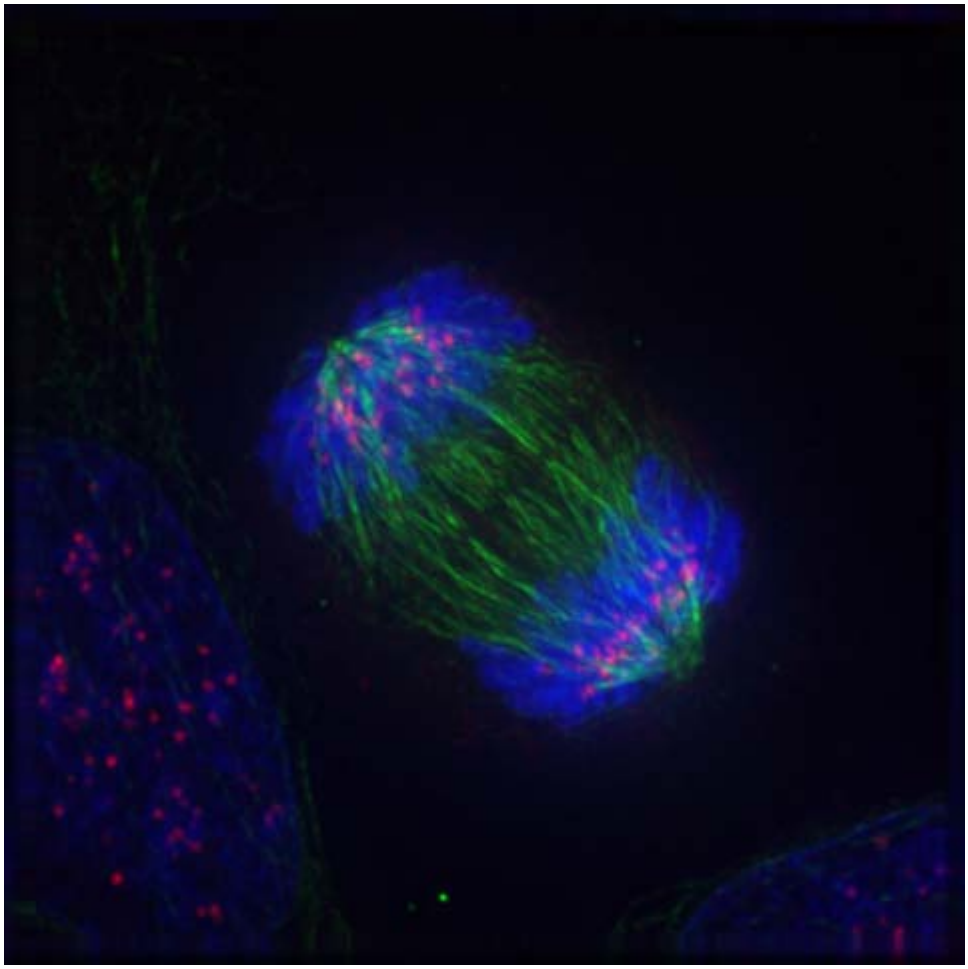
Early prophase: Nonkinetochore microtubules, shown as green strands, have established a matrix around the degrading nucleus, in blue. The green nodules are the centrosomes.



Early prometaphase: The nuclear membrane has just degraded, allowing the microtubules to quickly interact with the kinetochores on the chromosomes, which have just condensed.



Late metaphase: The centrosomes have moved to the poles of the cell and have established the mitotic spindle. The chromosomes, in light blue, have all assembled at the metaphase plate, except for one.



Anaphase: Lengthening nonkinetochore microtubules push the two sets of chromosomes further apart.

Chapter- 10

Homeostasis

Homeostasis (from Greek: ὅμοιος, *hómoios*, "similar" and στάσις, *stásis*, "standing still"; defined by Claude Bernard and later by Walter Bradford Cannon in 1926, 1929 and 1932) is the property of a system, either open or closed, that regulates its internal environment and tends to maintain a stable, constant condition. Typically used to refer to a living organism, the concept came from that of milieu interieur that was created by Claude Bernard and published in 1865. Multiple dynamic equilibrium adjustment and regulation mechanisms make homeostasis possible.

Biological

With regards to any given life system parameter, an organism may be a *conformer* or a *regulator*. On one hand, regulators try to maintain the parameter at a constant level over possibly wide ambient environmental variations. On the other hand, conformers allow the environment to determine the parameter. For instance, endothermic animals (mammals and birds) maintain a constant body temperature, while ectothermic animals (almost all other organisms) exhibit wide body temperature variation.

Behavioral adaptations allow ectothermic animals to exert some control over a given parameter. For instance, reptiles often rest on sun-heated rocks in the morning to raise their body temperature. Regulators are also responsive to external circumstances, however: if the same sun-baked boulder happens to host a ground squirrel, the animal's metabolism will adjust to the lesser need for internal heat production.

Thermal image of a cold-blooded tarantula (cold-blooded or ectothermic) on a warm-blooded human hand (endothermic).

An advantage of homeostatic regulation is that it allows an organism to function effectively in a broad range of environmental conditions. For example, ectotherms tend to become sluggish at low temperatures, whereas a co-located endotherm may be fully active. That thermal stability comes at a price since an automatic regulation system requires additional energy. One reason snakes may eat only once a week is that they use much less energy to maintain homeostasis.

Most homeostatic regulation is controlled by the release of hormones into the bloodstream. However, other regulatory processes rely on simple diffusion to maintain a balance.

Homeostatic regulation extends far beyond the control of temperature. Homeostasis requires the regulation of the pH of the Blood at 7.365 (a measure of alkalinity and acid). All animals also regulate their blood glucose, as well as the concentration of their blood. Mammals regulate their blood glucose with insulin and glucagon. The human body maintains glucose levels constant most of the day, even after a 24-hour fast. Even during long periods of fasting, glucose levels are reduced only very slightly. Insulin, secreted by the beta cells of the pancreas, effectively transports glucose to the body's cells by instructing those cells to keep more of the glucose for their own use. If the glucose inside the cells is high the cells will convert it to the insoluble glycogen to prevent the soluble glucose interfering with cellular metabolism. Ultimately this lowers blood glucose levels, and Insulin helps to prevent hyperglycemia. When insulin is deficient or cells become resistant to it, diabetes occurs. Glucagon, secreted by the alpha cells of the pancreas, encourages cells to break down stored glycogen or convert non-carbohydrate carbon sources to glucose via gluconeogenesis, thus preventing hypoglycemia. The kidneys are used to remove excess water and ions from the blood. These are then expelled as urine. The kidneys perform a vital role in homeostatic regulation in mammals, removing excess water, salt, and urea from the blood. These are the body's main waste products.

Another homeostatic regulation occurs in the gut. Homeostasis of the gut is not fully understood but it is believed that Toll-like receptor (TLR) expression profiles contribute to it. Intestinal epithelial cells exhibit important factors that contribute to homeostasis: 1) They have different cellular distribution of TLR's compared to the normal gut mucosa. An example of this is how TLR5 (activated by flagellin) can redistribute to the basolateral membrane, which is the perfect place where flagellin can be detected. 2) The enterocytes express high levels of TLR inhibitor Toll-interacting protein (TOLLIP). TOLLIP is a human gene that is a part of the innate immune system and is highest in a healthy gut; it correlates to luminal bacterial load. 3) Surface enterocytes also express high levels of Interleukin-1 receptor (IL-1R) -containing inhibitory molecule. IL-1R are also referred to as single immunoglobulin IL-1R (SIGIRR). Animals deficient in this are more susceptible to induced colitis, implying that SIGIRR might possibly play a role in tuning mucosal tolerance towards commensal flora. Nucleotide-binding oligomerisation domain containing 2 (NOD2) is suggested to have an effect on suppressing inflammatory cascades based on recent evidence. It is believed to modulate signals transmitted through TLRs, TLR3, 4, and 9 specifically. Mutation of it has resulted in Crohn's disease. Excessive T-helper 1 responses to resident flora in the gut are controlled by inhibiting the controlling influence of regulatory T cells and tolerance-inducing dendritic cells.

Sleep timing depends upon a balance between homeostatic sleep propensity, the need for sleep as a function of the amount of time elapsed since the last adequate sleep episode, and circadian rhythms that determine the ideal timing of a correctly structured and restorative sleep episode.

Control mechanisms

All homeostatic control mechanisms have at least three interdependent components for the variable being regulated: The receptor is the sensing component that monitors and responds to changes in the environment. When the receptor senses a stimulus, it sends information to a **control in the centre of it all metobolism center**, the component that sets the range at which a variable is maintained. The control center determines an appropriate response to the stimulus. In most homeostatic mechanisms the control center is the brain. The control center then sends signals to an effector, which can be muscles, organs or other structures that receive signals from the control center. After receiving the signal, a change occurs to correct the deviation by either enhancing it with positive feedback or depressing it with negative feedback

Positive feedback

Positive feedback is a mechanism by which an output is enhanced, such as protein levels. However, in order to avoid any fluctuation in the protein level, the mechanism is

inhibited stochastically (I), therefore when the concentration of the activated protein (A) is past the threshold ([I]), the loop mechanism is activated and the concentration of A increases exponentially if $d[A]=k[A]$

Positive feedback mechanisms are designed to accelerate or enhance the output created by a stimulus that has already been activated.

Unlike negative feedback mechanisms that initiate to maintain or regulate physiological functions within a set and narrow range, the positive feedback mechanisms are designed to push levels out of normal ranges. To achieve this purpose, a series of events initiates a cascading process that builds to increase the effect of the stimulus. This process can be beneficial but is rarely used by the body due to risks of the acceleration's becoming uncontrollable.

One positive feedback example event in the body is blood platelet accumulation, which, in turn, causes blood clotting in response to a break or tear in the lining of blood vessels. Another example is the release of oxytocin to intensify the contractions that take place during childbirth.

Negative feedback

Negative feedback mechanisms consist of reducing the output or activity of any organ or system back to its normal range of functioning. A good example of this is regulating blood pressure. Blood vessels can sense resistance of blood flow against the walls when blood pressure increases. The blood vessels act as the receptors and they relay this message to the brain. The brain then sends a message to the heart and blood vessels, both of which are the effectors. The heart rate would decrease as the blood vessels increase in diameter (or vasodilation). This change would cause the blood pressure to fall back to its normal range. The opposite would happen when blood pressure decreases, and would cause vasoconstriction.

Another important example is seen when the body is deprived of food. The body would then reset the metabolic set point to a lower than normal value. This would allow the body to continue to function, at a slower rate, even though the body is starving. Therefore, people who deprive themselves of food while trying to lose weight would find it easy to shed weight initially and much harder to lose more after. This is due to the body readjusting itself to a lower metabolic set point to allow the body to survive with its low supply of energy. Exercise can change this effect by increasing the metabolic demand.

Another good example of negative feedback mechanism is temperature control. The hypothalamus, which monitors the body temperature, is capable of determining even the slightest of variation of normal body temperature (37 degrees Celsius). Response to such variation could be stimulation of glands that produces sweat to reduce the temperature or signaling various muscles to shiver to increase body temperature.

Both feedbacks are equally important for the healthy functioning of one's body. Complications can arise if any of the two feedbacks are affected or altered in any way.

Homeostatic imbalance

Many diseases are a result of disturbance of homeostasis, a condition known as homeostatic imbalance. As it ages, every organism will lose efficiency in its control systems. The inefficiencies gradually result in an unstable internal environment that increases the risk for illness. In addition, homeostatic imbalance is also responsible for the physical changes associated with aging. Even more serious than illness and other characteristics of aging is death. Heart failure has been seen where nominal negative feedback mechanisms become overwhelmed, and destructive positive feedback mechanisms then take over.

Diseases that result from a homeostatic imbalance include diabetes, dehydration, hypoglycemia, hyperglycemia, gout, and any disease caused by a toxin present in the bloodstream. All of these conditions result from the presence of an increased amount of a particular substance. In ideal circumstances, homeostatic control mechanisms should prevent this imbalance from occurring, but, in some people, the mechanisms do not work efficiently enough or the quantity of the substance exceeds the levels at which it can be managed. In these cases, medical intervention is necessary to restore the balance, or permanent damage to the organs may result.

Varieties

The Dynamic Energy Budget theory for metabolic organization delineates structure and (one or more) reserves in an organism. Its formulation is based on three forms of homeostasis:

- **Strong homeostasis**, whereas structure and reserve do not change in composition. Because the amount of reserve and structure can vary, this allows a particular change in the composition of the whole body (as explained by the Dynamic Energy Budget theory).
- **Weak homeostasis**, wherein the ratio of the amounts of reserve and structure becomes constant as long as food availability is constant, even when the organism grows. This means that the whole body composition is constant during growth in constant environments.
- **Structural homeostasis**, wherein the sub-individual structures grow in harmony with the whole individual; the relative proportions of the individuals remain constant.

Ecological

The concept of homeostasis is central to the topic of Ecological Stoichiometry. There it refers to the relationship between the nutrient content of a resource and the nutrient

content of its resources. Stoichiometric homeostasis helps explain nutrient recycling and population dynamics.

Historically, ecological succession was seen as having a stable end-stage called the climax, sometimes referred to as the 'potential biodiversity' of a site, shaped primarily by the local climate. This idea has been largely abandoned by modern ecologists in favor of nonequilibrium ideas of how ecosystems function, as most natural ecosystems experience disturbance at a rate that makes a "climax" community unattainable.

Only on small, isolated habitats known as ecological islands can the phenomenon be observed. One such case study is the island of Krakatoa after its major eruption in 1883: the established stable homeostasis of the previous forest climax ecosystem was destroyed, and all life was eliminated from the island. In the years after the eruption, Krakatoa went through a sequence of ecological changes in which successive groups of new plant or animal species followed one another, leading to increasing biodiversity and eventually culminating in a re-established climax community. This *ecological succession* on Krakatoa occurred in a number of stages; a *sere* is defined as "a stage in a sequence of events by which succession occurs". The complete chain of seres leading to a climax is called a *prisere*. In the case of Krakatoa, the island reached its climax community, with eight hundred different recorded species, in 1983, one hundred years after the eruption that cleared all life off the island. Evidence confirms that this number has been homeostatic for some time, with the introduction of new species rapidly leading to elimination of old ones. The evidence of Krakatoa, and other disturbed island ecosystems, has confirmed many principles of Island Biogeography, mimicking general principles of ecological succession albeit in a virtually closed system comprised almost exclusively of endemic species.

Biosphere

In the Gaia hypothesis, James Lovelock stated that the entire mass of living matter on Earth (or any planet with life) functions as a vast homeostatic superorganism that actively modifies its planetary environment to produce the environmental conditions necessary for its own survival. In this view, the entire planet maintains homeostasis. Whether this sort of system is present on Earth is still open to debate. However, some relatively simple homeostatic mechanisms are generally accepted. For example, when atmospheric carbon dioxide levels rise, certain plants are able to grow better and thus act to remove more carbon dioxide from the atmosphere. When sunlight is plentiful and atmospheric temperature climbs, the phytoplankton of the ocean surface waters thrive and produce more dimethyl sulfide, DMS. The DMS molecules act as cloud condensation nuclei, which produce more clouds, and thus increase the atmospheric albedo, and this feeds back to lower the temperature of the atmosphere. As scientists discover more about Earth, vast numbers of positive and negative feedback loops are being discovered, that, together, maintain a metastable condition, sometimes within very broad range of environmental conditions. Environmental pressure, such as competition or change in temperature, can lead to adaptation/extinction of species over time.

Reactive

Example of use: "Reactive homeostasis is an immediate homeostasic response to a challenge such as predation."

However, *any* homeostasis is impossible without reaction - because homeostasis is and must be a "feedback" phenomenon.

The phrase "reactive homeostasis" is simply short for "reactive compensation reestablishing homeostasis", that is to say, "reestablishing a point of homeostasis." - it should not be confused with a separate *kind* of homeostasis or a distinct phenomenon *from* homeostasis; it is simply the compensation (or compensatory) phase of homeostasis.

Other fields

The term has come to be used in other fields, as well.

Risk

An actuary may refer to *risk homeostasis*, where (for example) people that have anti-lock brakes have no better safety record than those without anti-lock brakes, because the former unconsciously compensate for the safer vehicle via less-safe driving habits. Previous to the innovation of anti-lock brakes, certain maneuvers involved minor skids, evoking fear and avoidance: now the anti-lock system moves the boundary for such feedback, and behavior patterns expand into the no-longer punitive area. It has also been suggested that ecological crises are an instance of risk homeostasis in which a particular behavior continues until proven dangerous or dramatic consequences actually occur.

Stress

Sociologists and psychologists may refer to *stress homeostasis*, the tendency of a population or an individual to stay at a certain level of stress, often generating artificial stresses if the "natural" level of stress is not enough.

Jean-François Lyotard, a postmodern theorist, has applied this term to societal 'power centers' that he describes as being 'governed by a principle of homeostasis,' for example, the scientific hierarchy, which will sometimes ignore a radical new discovery for years because it destabilises previously-accepted norms.

Psychological

Author George Leonard discusses in his book *Mastery* how homeostasis affects our behavior and who we are. He states that homeostasis will prevent our body from making drastic changes and maintain stability in our lives even if it is detrimental to us. Examples

include when an obese person starts exercising, homeostasis in the body resists the activity to maintain stability. Another example Leonard uses is a unstable family where the father has been a raging alcoholic and suddenly stops and the son starts up a drug habit to maintain stability in the family. Homeostasis is the main factor that stops people changing their habits because our bodies view change as dangerous unless it is very slow. Leonard discusses this dilemma as the media today only encourages fast change and quick results. The opening of his book aptly describes his despair with the current state of the world and how it is at war with homeostasis. "The trouble is that we have few, if any, maps to guide us on the journey or even to show us how to find the path. The modern world, in fact, can be viewed as a prodigious conspiracy against mastery. We're continually bombarded with the promises of immediate gratification, instant success, and fast, temporary relief, all of which lead in exactly the wrong direction."