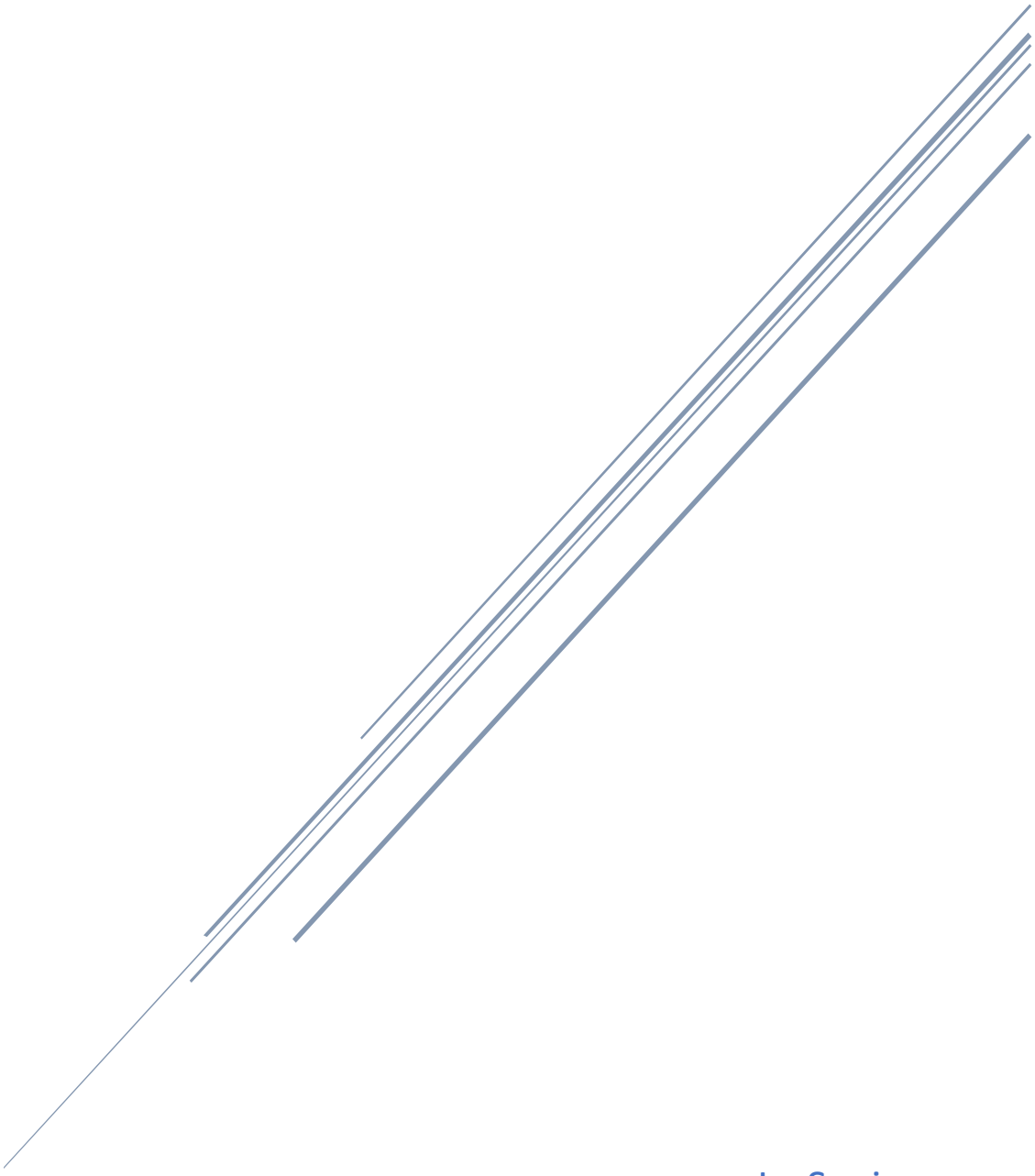
CELL BIOLOGY SEMESTER 2

Bioinformatics 2020



La Sapienza

8. Control of gene expression

An overview of gene expression

A typical eukaryotic cell expresses only a fraction of it genes, and the distinct types of cells in multicellular organisms arise because different sets of genes are expressed as a cell differentiates. So, the different cell types of a multicellular organism contain the same DNA and, therefore all the genetic instructions necessary to direct the formation of a complete organism. Hence, the cells of an organism differ not because they contain different genes, but because they express them differently.

Although all of the steps involved in expressing a gene can in principle be regulated, for most genes the initiation of transcription is the most important point of control. Thus a cell can control the proteins it makes by:

- Controlling when and how often a given gene is transcribed
- Controlling how the primary RNA transcript is spliced or otherwise processed
- Selecting which mRNAs are translated by ribosomes
- Selectively activating or inactivating proteins after they have been made

How transcription switches work

We saw earlier that the promoter region of a gene attracts the enzyme RNA polymerase and correctly orients the enzyme to begin its task of making an RNA copy of the gene. The promoters of both bacterial and eukaryotic genes include an initiation site, where transcription begins. In addition to the promoter, nearly all genes have regulatory DNA sequences that are used to switch the gene on or off. Regulatory DNA sequences do not work by themselves; to have any effect these sequences must be recognized by proteins called gene regulatory proteins that bind to the DNA. It is the combination of a DNA sequence and its associated protein molecules that acts as the switch to control transcription.

Although each gene regulatory protein has unique features, most bind to DNA using one of a small number of protein structure motifs. The precise amino acid sequence that is folded into the DNA-binding motif determines the particular DNA sequence that is recognized. The DNA-binding motifs are the homeodomain that consists of three linked α helices; the zinc finger that is built from an α helix and a β sheet held together by a molecule of zinc; and the leucine zipper that is formed by two α helices.

Repressors turn genes off, activators turn them on

RNA polymerase binds to the DNA and initiates transcription at a site called promoter. However, within the promoter is a short DNA sequence that is recognized by a gene regulatory protein. When the regulatory protein binds to this nucleotide sequence, termed the operator, it blocks access of RNA polymerase to the promoter. This prevents transcription of the operon and production of the tryptophan-producing enzymes; it switches genes off. The gene regulatory protein is known as the tryptophan repressor, and it is regulated in a clever way: the repressor can bind only to DNA if it has also bound several molecules of the amino acid tryptophan. Therefore, the tryptophan repressor in an allosteric protein: the binding of tryptophan causes subtle change in its three-dimensional structure so that it can now bind to the operator DNA.

Other bacterial gene regulatory proteins operate in the opposite manner by switching genes on, or activating them. These activator proteins bind to a regulatory sequence on the DNA and then interacts with the RNA polymerase to help it initiate transcription. Without the activator, the promoter fails to initiate transcription efficiently.

Differences in regulation of transcription

Regulation of transcription in eucaryotes differs in four important ways from that in bacteria:

1. While bacteria contain a single type of RNA polymerase, eukaryotic cells have three: RNA polymerase I / II / III. These polymerases are responsible for transcribing different types of genes. RNA polymerases III and I transcribe the genes encoding tRNA, rRNA and small RNAs. RNA polymerase II transcribes the vast majority of eukaryotic genes.
2. Bacterial RNA polymerase is able to initiate transcription without the help of additional proteins. However, eucaryotic RNA polymerases require the assembly of proteins called general transcription factors. The general are thought to position the RNA polymerase correctly at the promoter, to aid in pulling apart the two strands of DNA to allow transcription to begin, and to allow RNA polymerase to leave the promoter as transcription begins.
3. In bacteria, regulatory proteins usually bind to regulatory DNA sequences close to where RNA polymerase binds and then either activate or repress transcription of the gene. In eucaryotes, these regulatory DNA sequences are often separated from the promoter by many thousands of nucleotide pairs. So, in eucaryotes gene activation occurs at a distance.
4. Initiation of transcription in eukaryotic cells must also take into account the packing of DNA into nucleosomes and more compact forms of chromatin structure.

Eucaryotic gene regulatory proteins act in two fundamental ways:

1. They can directly affect the assembly process of RNA polymerase and the general transcription factors at the promoter
2. They can locally modify the chromatin structure of promoter regions

The molecular mechanisms that create specialized cell types

In eucaryotes, the expression of a gene is generally controlled by a combination of gene regulatory proteins. The regulatory proteins do not each function individually, but they work together as a ‘committee’ to control gene expression.

In multicellular plants and animals, the production of different gene regulatory proteins in different cell types ensures the expression of only those genes appropriate to the particular type of cell. Although all cells must be able to switch genes on and off, multicellular organisms require special gene switching mechanisms for generating and maintaining their different types of cells. Once a cell in a multicellular organism has become differentiated into a particular cell type, it will generally remain differentiated, and if it is able to divide, all it progeny cells will be of that same cell type. This means that the changes in gene expression that give rise to a differentiated cell must be remembered and passed on to its daughter cells through all subsequent cell divisions. Cells have several ways of ensuring that daughter cells remember what kind of cells they are supposed to be:

1. Trough a positive feedback loop, where a key gene regulatory protein activates transcription of its own gene in addition to that of other cell-type-specific genes
2. Trough the propagation of a condensed chromatin structure from parent to daughter cell even though DNA replication intervenes

A single gene regulatory protein, if expressed in the appropriate precursor cell, can trigger the formation of a specialized cell type or even an entire organ.

9. How genes and genomes evolve

The vast diversity of life we see around us has arisen through changes in DNA sequences that have accumulated since the first cells on earth arose some 3.5 billion years ago.

Generating genetic variation

Genetic changes that offer an organism a selective advantage or those that are selectively neutral are the most likely to be perpetuated. Changes that seriously compromise an organism's fitness are eliminated through natural selection.

Genetic variation (the raw material for evolutionary change) occurs by a variety of mechanisms and each of these forms of genetic variation has played an important part in the evolution of modern organisms:

1. *Mutations within a gene:* an existing gene can be modified by mutations that change a single nucleotide or that delete or duplicate on or more nucleotides in its DNA sequence. These so called point mutations typically arise from small errors in DNA replication or repair.
2. *Gene duplication:* an existing gene, a larger segment of DNA, or even a whole genome can be duplicated, creating a set of closely related genes within a single cell. Gene duplication is one of the most important sources of genetic diversity. Once a gene has been duplicated, one of the two gene copies is free to mutate and become specialized to perform a different function. Repeated rounds of this process of duplication and divergence can allow one gene to give rise to a whole family of genes within a single genome.
3. *Gene deletion:* individual genes, or whole blocks of genes can be lost through chromosome breakage and failures of repair.
4. *Exon shuffling:* the evolution of new proteins is thought to have been greatly facilitated by the organization of eukaryotic genes as relatively short exons separated by long, noncoding introns. The presence of introns greatly increases the probability that a chance recombination event generate a functional hybrid gene by joining together two initially separate exons coding for quite different protein domains; this process called exon shuffling.
5. *Horizontal (intracellular) gene transfer:* a piece of DNA can be transferred from the genome of one cell to that of another, even to that of another species. This process is rare among eucaryotes, but common among procaryotes.

Reconstructing life's family tree

By comparing the nucleotide or amino acid sequences of contemporary organisms, we are beginning to able to reconstruct how genomes have evolved in the billions of years that elapsed since the appearance of the first cells.

Examining the human genome

The human genome contains 3.2 x 10^9 nucleotide pairs divided among 22 autosomes and 2 sex chromosomes. The human genome sequence refers to the complete nucleotide sequence of the DNA contained in these 24 chromosomes.
Individual human differ from one another by an average of 1 nucleotide pair in every 1000; this variation underlies our individuality and provides the basis for identifying individuals by DNA analysis.

The first characteristic feature of the human genome is how little of it (only a few percent) codes for proteins or for structural or catalytic RNAs. Much of the remaining DNA is made up of transposable elements that have gradually colonized our genome over evolutionary time. A second feature of the human genome is the very large average size of 27.000 nucleotide pairs. Only about 1300 nucleotide pairs are required to encode a protein of average size, and most of the remaining DNA is a gene consists of long stretches of noncoding DNA that interrupt the relatively short protein-coding exons. Finally, the nucleotide sequence of the human genome has revealed that the critical information it carries seems to be in an alarming state of disarray.

A major obstacle in interpreting the nucleotide sequences of human chromosomes is the fact that much of the sequence appears unimportant. Comparative genome analyses provide a valuable tool for indentifying genes as well as functionally important regulatory sequences. Knowing the location, and possibly the function, of a gene in one genome consequently makes it easier to identify and predict the function of the corresponding gene in the other genome. Such comparisons have revealed that mice and humans share most of the same genes, and that large blocks of the mouse and human genomes contain these genes in the same order.

Even with the human genome in hand, many questions will continue to challenge cell biologists throughout the next century. Perhaps most puzzling is to determine how organisms built from essentially the same set of proteins can be so different. This will require understanding how genes are regulated and alternatively spliced to define each organism's developmental programs.

15. Intracellular compartments and transport

Membrane-enclosed organelles

Whereas a prokaryotic cell consists of a single compartment, the cytosol, enclosed by the plasma membrane, a eukaryotic cell is subdivides by internal membranes. These membranes create enclosed comportments in which sets of enzymes can operate without interference from reactions occurring in other compartments. They major membrane-enclosed organelles are:

- *Nucleus:* this is generally the most prominent organelle in eukaryotic cells. It is surrounded by a double membrane, known as the nuclear envelope, and communicates with the cytosol via nuclear pores that perforate the envelope.
- *Endoplasmatic reticulum (ER):* a system of interconnected sacs and tubes of membrane that often extends throughout most of the cell. The ER is the major site of new-membrane synthesis in the cell. Large areas of the system have ribosomes attached to the cytosolic surface and are called rough ER. The ribosomes are synthesizing proteins that are delivered into the ER lumen or ER membrane. The smooth ER is the site of steroid hormone synthesis and the site where a variety of organic molecules (including alcohol) are detoxified.
- *Golgi apparatus:* receives proteins and lipids from the ER, modifies them, and then dispatches them to other destinations in the cell.
- *Lysosomes:* small sacs of digestive enzymes that degrade worn-out organelles, as well as macromolecules and particles taken into the cell by endocytosis.
- *Endosomes:* sort the ingested molecules and recycle some back to the plasma membrane.
- *Mitochondria:* their main function is ATP synthesis by oxidative phosphorylation
- *Chloroplasts (in plant cells):* by photosynthesis they produce ATP and carbon fixation
- *Peroxisomes:* they contain enzymes that break down lipids and destroy toxic molecules.

Much can be learned about the composition and function of an organelle once it has been isolated from other cell structures; organelles are isolated by differential centrifugation. Many of the membrane-enclosed organelles, including the ER, Golgi apparatus, mitochondria, and chloroplasts, are held in their relative locations in the cell by attachment to the cytoskeleton, especially to microtubules.

To understand the relationship between the different compartments of a modern eukaryotic cell, it is useful to consider how they might have evolved. The precursors of the first eukaryotic cells are thought to have been simple microorganisms, which had a plasma membrane but no internal membranes. The plasma membrane in such cells would have provided all membrane-dependent functions, as does a plasma membrane in most bacteria. Membrane-enclosed organelles are thought to have arisen in evolution in at least two ways:

1. The nuclear membrane and the membranes of the ER, Golgi apparatus, endosomes, and lysosomes are believed to have originated by invagination of the plasma membrane. These membranes, and the organelles they enclose, are all part of what is called the endomembrane system.
2. Mitochondria and chloroplasts differ from all other organelles in that they possess their own small genomes and can make some of their own proteins. The similarity of these genomes to those of bacteria suggests that mitochondria and chloroplasts evolved from bacteria that were engulfed by primitive eukaryotic cells with which they initially lived in symbiosis. This would also explain why these organelles are enclosed by two membranes.

Protein sorting

Before a eukaryotic cell reproduces by dividing in two, it has to duplicates its membrane-enclosed organelles. As a cell grows, membrane-enclosed organelles enlarge by incorporation of new molecules. The organelles then divide and at cell division they are distributed between the two daughter cells. The nuclear envelope, ER and Golgi apparatus break up into small vesicles, which ten coalesce again as the two daughter cells are formed. Organelle growth requires a supply of new lipids to make more membrane and a supply of the appropriate proteins. These newly synthesized proteins must be accurately delivered to organelles.

The synthesis of all proteins in the cell begins of ribosomes in the cytosol. The exceptions are the few mitochondrial and chloroplasts proteins that are synthesized on ribosomes inside these organelles. However, most mitochondrial and chloroplasts proteins are made in the cytosol. The fate of any protein molecule synthesized in the cytosol depends on its amino acid sequence, which can contain a sorting signal that directs the protein to the organelle in which it is required. Proteins that lack such signals remain as permanent residents in the cytosol. The typical sorting signal on proteins is a stretch of amino acids sequence, 15-60 amino acids long. This signal sequence is often removed from the finished protein once the sorting decision has been executed.

Proteins are imported into the organelles by three mechanisms:

1. Nuclear proteins contain nuclear localization signals that help direct their active transport from the cytosol into the nucleus through nuclear pores, which penetrate the double-membrane nuclear envelope. First, specialized proteins, called nuclear transport receptors, bind to the prospective nuclear protein. The resulting complex is guided to a nuclear pore by fibrils at extend from the pore into the cytosol. The binding of the nuclear proteins to the pore, opens the pore, and the complex is actively transported into the nucleus by a process that uses the energy provided by GTP hydrolysis. The receptors are then exported back through the pores into the cytosol for reuse. Proteins can enter the nucleus without being unfolded.
2. Most mitochondrial and chloroplast proteins are made in the cytosol and are then actively transported into the organelles by protein translocators in their membranes. Proteins must be unfolded to allow them to snake through the chloroplast or mitochondrial membrane. Chaperone proteins inside the organelles help to pull the proteins across the two membranes and to refold the protein once it is inside. Subsequent transport to a particular site within the organelle usually requires further sorting signals in the protein, which are often only exposed after the first signal sequence in removed. Proteins moving from cytosol into the ER also use the mechanism of protein translocators.
3. Proteins moving from the ER onward and from one compartment of the endomembrane system to another are transported by transport vesicles.

The outer nuclear membrane is continuous with the ER. The ER is the membrane factory of the cell; it makes most of the cell's lipids and many of its proteins. The proteins are made by ribosomes bound to the surface of the rough ER. There are two separate populations of ribosomes in the cytosol. Membrane-bound ribosomes are attached to the cytosolic side of the ER membrane and are making proteins that are being translocated into the ER. Free ribosomes are unattached to any membrane and are making al of the other proteins encoded by nuclear DNA.

Ribosomes in the cytosol are directed to the ER if the protein they are making has an ER signal sequence, which is recognized by a signal-recognition particle (SRP) in the cytosol; the binding of the ribosome-SRP complex to a receptor on the ER membrane initiates the translocation process that threads the growing polypeptide across the ER membrane through a translocation channel. Soluble proteins destined for secretion or the lumen of an organelle pass completely into the ER lumen, while transmembrane proteins destined for the ER membrane or other cell membrane remain anchored in the lipid bilayer by one or more membrane-spanning a helices.

In the ER lumen, proteins fold up, assemble with other proteins, form disulfide bonds, and become decorated with oligosaccharide chains. Exit from the ER is an important quality-control step; proteins that either fail to fold properly or fail to assemble with their normal partners are retained in the ER and are eventually degraded.

Vesicular transport

Protein transport from the ER to the Golgi apparatus and from the Golgi apparatus to other destinations is mediated by transport vesicles that continually bud off from one membrane and fuse with another, a process called vesicular transport.

The vesicular traffic between membrane-enclosed compartments of the endomembrane system is highly organized. In the outward secretory pathway protein molecules are transported from the ER, through the Golgi apparatus, to the plasma membrane or lysosomes. In the inward endocytic pathway extracellular molecules are ingested in vesicles derived from the plasma membrane and are delivered to early endosomes and then to lysosomes.

Vesicles that bud from membranes usually have a distinctive protein coat on their cytosolic surface and are therefore called coated vesicles. After budding from its parent organelle, the vesicle sheds it coat, allowing its membrane to interact directly with the membrane to which it will fuse. Cells produce several kinds of coated vesicles, each with a distinctive protein coat; the assembly of the coat drives the budding process, and the coat proteins help incorporate receptors with their bound cargo molecules into the forming vesicle.
Best-studied vesicles are those that have coats made of the protein clathrin. These clathrin-coated vesicles bud from the Golgi apparatus on the outward secretory pathway and from the plasma membrane on the inward endocytic pathway. Clathrin molecules form basketlike cages that help shape membranes into vesicles. Clathrin itself plays no part in capturing specific molecules for transport. This is the function of a second class of coat proteins called adaptins, which both secure the clathrin coat to the vesicle membrane and help select cargo molecules for transport. Molecules for onward transport carry specific transport signals that are recognized by cargo receptors in the compartment membrane. Adaptins help capture specific cargo molecules by trapping the cargo receptors that bind them. In this way a selected set of cargo molecules, bound to their specific receptors, is incorporated into the lumen of each newly formed cathrin-coated vesicle.

Coated vesicles lose their protein coat soon after pinching off, enabling them to dock and fuse with a particular target membrane. Docking and fusion are thought to be mediated by proteins on the vesicle and on the target membranes, called v-SNAREs and t-SNAREs, respectively. Each organelle and each type of transport vesicle is believed to carry a unique SNARE, and the interactions between complementary SNAREs help ensure that transport vesicles fuse only with the correct membrane. Fusion not only delivers the contents of the vesicle into the interior of the target organelle, it also adds the vesicle membrane to the membrane of the organelle. Pairing of v-SNAREs and t-SNAREs forces the two lipid bilayers into close apposition. Lipids then flow between the two bilayers and the membranes fuse.

Secretory pathways

The Golgi apparatus receives newly made proteins from the ER; it modifies their oligosaccharides, sort proteins, and dispatches them from the trans Golgi network to the plasma membrane, lysosomes, or secretory vesicles. Some proteins are destined to function in the ER and they are retained in the ER by a C-terminal sequence of four amino acids called an ‘ER retention signal’, which is recognized by a membrane-bound receptor protein in the ER and Golgi apparatus. However, most proteins that enter the ER are destined for other locations. As mentioned above, exit from the ER is highly selective; chaperones prevent misfolded or partially assembled proteins from leaving the ER.

The mutation that causes the genetic disease cystic fibrosis, which causes severe degeneration of the lung, produces a plasma-membrane transport protein that is slightly misfolded. The devastating disease results not because the mutation inactivates an important protein, but because the active protein is discharged by the cell before it is given an opportunity to function.

After the exit from the ER, proteins are further modified and sorted in the Golgi apparatus. The Golgi apparatus consists of a collection of flattened, membrane-enclosed sacs (cisternae). Each Golgi stack has two distinct faces: an entry (or cis) fase and an exit (or trans) face. The cis face is adjacent to the ER, while the trans face points toward the plasma membrane. Soluble proteins and membrane enter the cis Golgi network via transport vesicles derived from the ER. The proteins travel through the cisternae in sequence by means of transport vesicles that bud from one cisterna and fuse with the next. Proteins exit from the trans Golgi network in transport vesicles destined for either the cell surface or another compartment. Both the cis and trans Golgi network are thought to be important for protein soring.

In all eukaryotic cells, transport vesicles continually bud from the trans Golgi network and fuse with the plasma membrane, a process called constitutive exocytosis; the process delivers plasma membrane lipids and proteins to the cell surface and also releases molecules from the cell, a process called secretion. In addition to the constitutive exocytosis pathway, which operated continually, there is also a regulated exocytosis pathway, which operates only in cells that are specialized for secretion. Specialized secretory cells produce large quantities of particular products, such as hormones or digestive enzymes, which are stored in secretory vesicles for later release. These vesicles bud off from the trans Golgi network and accumulate near the plasma membrane. There they wait for the extracellular signal that will stimulate them to fuse with the plasma membrane and release their contents to the cell exterior.

Endocytic pathway

Cells ingest fluid, molecules, and sometimes even particles, by endocytosis, in which regions of plasma membrane invaginate and pinch off to form endocytic vesicles. Two main types of endocytosis are distinguished on the basis of the size of the endocytic vesicles formed:

1. Pinocytosis (‘cellular drinking’): this involves the ingestion of fluid and molecules via small vesicles (<150 nm in diameter)
2. Phagocytosis (‘cellular eating’): this involves the ingestion of large particles, such as microorganisms and cell debris, via large vesicles called phagosomes (>250 nm in diameter)

Whereas all eukaryotic cells are continually ingesting fluid and molecules by pinocytosis, large particles are ingested mainly by specialized phagocytic cells. Phagocytic cells ingest large particles by taking them up into phagosomes; these phagosomes then fuse with lysosomes. Phagocytosis is important for digesting food molecules and defense against infections by ingesting invading microorganisms. Because during pinocytosis, a cell’s total surface area and volume remain unchanged, it is clear that as much membrane is being added to the cell surface by vesicle fusion (exocytosis) as is being removed by endocytosis.

Much of the material that is endocytosed is delivered to endosomes and then to lysosomes, where hydrolytic enzymes degrade it; most of the components of the endocytic vesicle membrane, however, are recycled in transport vesicles back to the plasma membrane for reuse.

16. Cell communication

Cells in multicellular organisms communicate through a large variety of extracellular chemical signals. And communication involves converting information signals from one form to another, a process called signal transduction. In a typical communication between cells, the signaling cell produces a particular type of signal molecule that is detected by the target cell. The target cells possess receptor proteins that recognized and respond specifically to the signal molecule. Signal transduction begins when the receptor protein on the target cell receives an incoming extracellular signal and converts it to the intracellular signals that alter cell behavior.

General principles of cell signaling

Single cells and cells in multicellular organisms use a variety of extracellular molecules to send signals to one another:

1. Endocrine signals: hormones produced in endocrine glands are secreted into the bloodstream and are often distributed widely throughout the body
2. Paracrine signals: the signaling molecules diffuse locally through the extracellular medium, remaining in the neighborhood of the cell that secretes them. Thus they act as local mediators on nearby cells.
3. Neuronal signals: neurons can deliver messages across long distances, but in the case of neuronal signaling the message is not broadcast widely but is transmitted quickly along axons to remote target cells.
4. Contact-dependent signals: this style of signaling does not require the release of a secreted molecule. Instead, the cells make direct contact through signaling molecules lodged in their plasma membranes. The message is delivered when a signal molecule anchored in the plasma membrane of the signaling cell binds to a receptor molecule embedded in the plasma membrane of the target cell.

Many of the signal molecules that regulate inflammation at the site of an infection or control cell proliferation in a healing wound function as paracrine signals. In embryonic development contact-dependent signaling plays an important role; for example it controls nerve-cell production.

A cell in a multicellular organism is exposed to hundreds of different signal molecules in its environment. Each cell must respond selectively to this mixture of signals, disregarding some and reacting to others, according to the cell's specialized function. Whether a cell responds to a signal molecule depends first on whether is possesses a receptor for that signal. But one signal can still be used to control the behavior of the cell in complex ways:

1. One signal, binding to one type of receptor protein, can cause a multitude of effect in the same target cell. Furthermore, different types of cells respond to the same signal in different ways.
2. A cell possesses a collection of different receptors. Such variety makes the cell sensitive to many extracellular signals. These signal molecules work in combinations to regulate the behavior of the cell.

Cells are stimulated by an extracellular signal molecule when it binds to and activates a receptor protein. Each receptor protein recognizes a particular signal molecule. Receptor proteins act as transducers, converting signals from one physical form to another. However, most extracellular signal molecules cannot pass through the plasma membrane; first they must bind to cell-surface receptor proteins that transduce the extracellular signal into different intracellular signals.

Extracellular signal molecules fall into two classes:

1. Signal molecules that are too large are too hydrophilic to cross the plasma membrane of the target cell. They bind on receptors on the surface of the target cell to relay their message across the membrane.
2. Signal molecules that are small enough or hydrophobic enough to slip easily through the plasma membrane. Once inside, these signal molecules either activate intracellular enzymes or bind to intracellular receptor proteins that regulate gene expression. Steroid hormones (including cortisol and testosterone) and nitric oxide (NO) are examples of small hydrophobic extracellular signal molecules. Acetylcholine released by nerve terminals in the blood-vessel wall stimulates endothelial cells lining the blood vessel to make and release NO. The NO diffuses out of the endothelial cells and into adjacent smooth muscle cells, causing them to relax.

In contrast to NO and the steroid hormones, the majority of signal molecules are too large or hydrophilic to cross the plasma membrane of the target. These signal molecules bind to receptor proteins on the target cell surface:

There are three main classes of cell-surface receptors:

1. Ion-channel-linked receptors: this receptor opens in response to binding of its signal molecule. The result is a flow of ions across the membrane, which produces an electrical current

2. G-protein-linked receptors: when a G-protein-linked receptor binds its extracellular signal molecule, the signal is passes first to a GTP-binding protein (a G-protein) that is associated with the receptor. The activated G-protein then leaves the receptor and turns on a target enzyme in the plasma membrane
3. Enzyme-linked receptors: when binding of the signal molecule activates this receptor, enzyme activity is switched on at the other end of the receptor, which is inside the cell

In sum, G-protein-linked receptors and enzyme-linked receptors respond to extracellular signals by initiating cascades of intracellular signaling reactions that alter the behavior of the cell.

G-protein-linked receptors

G-protein-linked receptors form the largest family of cell-surface receptors. They mediate responses to an enormous diversity of extracellular signal molecules. These signal molecules are as varied in structure as they are in function. Despite the diversity of the signal molecules that bind to them, all G-protein-linked receptors possess a similar structure: each is made of a single polypeptide chain that threads back and forth across the lipid bilayer seven times. There are also several varieties of G proteins. All of these G proteins, however, have a similar general structure and operate in a similar way. All G proteins are composed of three protein subunits: the a, b and g subunits.

In the unstimulated state, the a subunit has GDP bound to it, and the G protein is purposeless. When an extracellular ligand binds to its receptor, the altered receptor activates a G protein by causing the a subunit to lose some of its affinity for GDP, which it exchanges for a molecule of GTP. This activation breaks up the G protein subunits: the switched-on a subunit detaches from the bg complex, giving rise to two separate molecules that now roam independently along the plasma membrane. The amount of time that the a- and bg subunits remain dissociated is limited by the behavior of the a subunit. The a subunit has an intrinsic GTP-hydrolyzing (GTPase) activity, and it eventually hydrolyzes its bound GTP back to GDP; the a subunit then reassociates with the bg complex. The reconstituted G protein is now ready to be reactivated by another activated receptor.

In concluding, when an extracellular signal molecule binds to a seven-pass transmembrane receptor, the receptor protein undergoes a conformational change that enables it to activate a G protein located on the underside of the plasma membrane. These G-proteins act as molecular switches, transmitting the signal onward for a short period and then switching themselves off by hydrolysis their bound GTP and GDP. This system demonstrates that the mechanisms that shut a signal off are as important as the mechanisms that turn it on. The disease cholera is caused by a bacterium that multiplies in the intestine where it produces a protein called cholera toxin. This protein enters the cells that line the intestine and modifies the a subunit of a G-protein in such way that it can no longer hydrolyze its bound GTP. The altered a subunit thus remains in the active state, continuously transmitting a signal to its target proteins. In intestinal cells, this causes a prolonged and excessive outflow of Cl- and water into the gut, resulting in catastrophic diarrhea and dehydration.

As mentioned above, there are many different G proteins:

- Some G proteins directly regulate ion channels in the plasma membrane. For example, G proteins couple receptor activation to the opening of K+ channels in the plasma membrane of heart muscle cells.
- Other G proteins activate membrane-bound enzymes. The most frequent target enzymes for G proteins are adenylyl cyclase, the enzyme responsible for production of the signaling molecule cyclic AMP, and phospholipase C, which generates the messenger molecules inositol trisphosphate (IP3) and diacylglycerol.

We take a closer look to G proteins that activate membrane-bound enzymes. Adenylyl cyclase and phospholipase C are activated by different types of G proteins, so that cells are able to couple production of the signaling molecules to different extracellular signals. Many extracellular signals acting via G protein-linked receptors affect the activity of adenyl cyclase. Most commonly, the activated G protein a subunit switches on

the adenyl cyclase, causing an increase in the synthesis of cyclic AMP from ATP. To help eliminate the signal, a second enzyme, called cyclic AMP phoshodiesterase, converts cyclic AMP to ordinary AMP. Cyclic AMP exerts various effects by activating the enzyme protein kinase A (PKA). This enzyme is normally held inactive in a complex with another protein. The binding of cyclic AMP forces a conformational change that unleashes the active kinase. In some cases the effects of activating a cyclic AMP cascade are rapid; in others the effects are slow. A rise in intracellular cyclic AMP can activate gene transcription.

The inositol phospolipid pathway begins with the activation of the membrane-bound enzyme phospholipase C. This enzyme activates two signaling pathways: IP3 and diacylglycerol. IP3 opens ion channels in the membrane of the endoplasmatic reticulum, releasing a flood of free Ca2+ ions into the cytosol. Ca2+ itself acts as an intracellular messenger, altering the activity of a wide range of proteins. Together with this Ca2+, diacylglycerol helps recruit and activate an enzyme called protein kinase C (PKC).

In concluding, a rise in cyclic AMP activates protein kinase A (PKA), while CA2+ and diacylglycerol in combination activate protein kinase C (PKC). PKA and PKC phosphorylate secreted target proteins on serines and threonines, thereby altering protein activity. Different cell types contain different sets of target proteins and are affected in different ways.

In general, stimulation of G-proteins-linked receptors produces rapid and reversible cell responses!

Enzyme-linked receptors

Plants, like animals use enzyme-linked cell-surface receptors to control their growth and development. Like G-protein-linked receptors, enzyme-linked receptors are transmembrane proteins that display their ligand-binding domains on the outer surface of the plasma membrane. Instead of associating with a G protein, the cytoplasmatic domain of the receptor acts as an enzyme or forms a complex with another protein that acts as an enzyme. Most are receptor tyrosine kinase, which phosphorylate tyrosines on selected intracellular proteins. Activated receptor tyrosine kinases cause the assembly of an intracellular signaling complex on the intracellular tail of the receptor. A part of this complex serves to activate Ras, a small GTP-binding protein, which activates a cascade of protein kinases that relay the signal from the plasma membrane to the nucleus. This cascade is called a MAP-kinase cascade.

MAP-kinase is the final kinase in the chain and it phosphorylates certain gene regulatory proteins on serines and threonines, altering their ability to control gene transcription and thereby causing a change in the pattern of gene expression. This shift may stimulate cell proliferation, cell survival or induce cell differentiation. However, mutations that stimulate cell proliferation by making Ras constantly active are a common feature of many cancers.

Some enzyme-linked receptors activate a direct pathway to the nucleus. Instead of activating signaling cascades, they turn on gene regulatory proteins right at the plasma membrane. Unlike the receptor tyrosine kinases that stimulate signaling cascades, cytokine receptors have no intrinsic enzyme activity. Instead, they are associated with cytoplasmatic tyrosine kinases called JAKs that are activated when a cytokine binds to its receptor. Once activated, the JAKs phosphorylate and activate cytoplasmatic gene regulatory proteins called STATs, which then migrates to the nucleus, where they stimulate transcription of specific target genes.
An even more direct signaling pathway is used by the TGF-b receptors, which activate gene regulatory proteins directly at the plasma membrane.

Different intracellular signaling pathways interact, enabling cells to produce an appropriate response to a complex combination of signals. Some combinations of signals will cause it to proliferate; and in the absence of any signals, most cells will kill themselves by undergoing apoptosis.

17. Cytoskeleton

The cytoskeleton is an intricate network of protein filaments that extend throughout the cytoplasm. It is responsible for large-scale movements, and without the cytoskeleton, wounds would never heal, muscles would be useless, and sperm would never reach the egg. The cytoskeleton is build on a framework of three types of protein filaments, and each type of filament has distinct mechanical properties and is formed from a different protein subunit.

1. *Intermediate filaments*: these are ropelike fibers made of intermediate filament proteins. One type of intermediate filaments forms a meshwork called the nuclear lamina just beneath the inner nuclear membrane. Other types extend across the cytoplasm, giving cells mechanical strength and distributing the mechanical stresses in an epithelial tissue by spanning the cytoplasm from one cell to another.
2. *Microtubules*: these are long, hollow cylinders made of the protein tubulin. They are more rigid than actin filaments or intermediate filaments. Microtubules have typically one end attached to a single microtubule-organizing center called a centrosome.
3. *Actin filaments*: these are flexible structures made of the protein actin. Although actin filaments are dispersed throughout the cell, they are most highly concentrated in the cortex (the layer of cytoplasm just beneath the plasma membrane)

Intermediate filaments

The main function of intermediate filaments is to enable cells to withstand the mechanical stress that occurs when cells are stretched. Intermediate filaments are therefore present along the length of nerve cell axons, in muscle cells and in epithelial cells. In all these cells, intermediate filaments, by stretching and distributing the effect of locally applied forces, keep the cells and their membranes from breaking.

The filaments are called 'intermediate' because their diameter (10nm) is between that of the thin actin-containing filaments and the ticker myosin filaments of smooth muscle cells. Intermediate filaments are the toughest and most durable of the three types of cytoskeletal filaments. The intermediate filaments are found in the cytoplasm of most animal cells. They typically form a network throughout the cytoplasm, surrounding the nucleus and extending out to the cell periphery. Furthermore, intermediate filaments are also found within the nucleus as the nuclear lamina, which underlies and strengthens the nuclear envelope.
Intermediate filaments can be grouped intro four classes:

1. Keratin filaments in epithelial cells. Keratin filaments typically span the interiors of epithelial cells from one side to the cell to the other, and filaments in adjacent epithelial cells are indirectly connected through cell-cell junctions called desmosomes. This network distributes the stress that occurs when the skin is stretched. The importance of this function is illustrated by the rare genetic disease epidermolysis bullosa simplex, in which mutations in the keratin genes interfere with the formation of keratin filaments in the epidermis. As a result, the skin is highly vulnerable to mechanical injury, and even a gentle pressure can rupture its cell, causing the skin to blister.
2. Vimentin and vimentin-related filaments in connective-tissue, muscle and neuroglial cells
3. Neurofilaments in nerve cells
4. Nuclear lamins, which strengthen the nuclear membrane of all animal cells

Filaments of each class are formed by polymerization of their corresponding protein subunits. Plectin aids in the bundling of intermediate filaments and links these filaments to other cytoskeletal protein networks. Mutations in the gene for plectin cause a devastating disease that combines features of epidermolysis bullosa simplex, muscular dystrophy and neurodegeneration. Thus, although plectin may not be necessary for the initial formation of intermediate filaments, its cross-linking action is crucial to withstand the mechanical stress.

Microtubules

Microtubules are stiff, hollow tubes formed by polymerization of tubulin dimer subunits. They are polarized structures with a slow-growing 'minus' end (the a-tubulin end) and a fast-growing 'plus' end (the b-tubulin end). Microtubules are nucleated in, and grow out from, organizing centers such as the centrosome. The minus ends of the microtubules are embedded in the organizing center. Centrosomes contain hundreds of ring-shaped structured formed from another type of tubuline, g-tubulin, and each g-tubulin ring serves as the starting point (or nucleation site) for the growth of one microtubule.

Once a microtubule has been nucleated it has in a labile, dynamic state in which the microtubule alternate between a growing state and a shrinking state. These transitions, known as dynamic instability, are controlled by the hydrolysis of GTP bound to tubulin dimers. Each tubulin dimer has a tightly bound GTP molecule that is hydrolyzed to GDP after the tubulin assembles into a microtubule. GTP hydrolysis reduces the affinity of the subunit for its neighbors and decreases the stability of the polymer, causing it to dissemble. In this way, GTP hydrolysis controls the growth of microtubules.

The relative instability of microtubules allows them to undergo continual rapid remodeling, and this is crucial for microtubule function, as demonstrated by the effect of drugs that prevent polymerization or depolymerization of tubulin. If a cell in mitosis is exposed to the drug colchicine, which binds tightly to free tubulin and prevents its polymerization into microtubules, the mitotic spindle rapidly disappears and the cell is unable to partition its chromosomes into two groups. This shows that the mitotic spindle is normally maintained by a continuous balanced addition and loss of tubulin subunits. The inactivation or destruction of the mitotic spindle will eventually kill dividing cells.

Cells are able to modify the dynamic instability of their microtubules for particular purposes. When a cell has differentiated into a specialized cell type and taken on a definite fixed structure, the dynamic instability of its microtubules or along their length and stabilize them against disassembly. The stabilized microtubules then serve to maintain the organization of the cell. Proteins that capture the plus end can stabilize microtubules. Its important to realize that the microtubules in living cells do not act alone.

Intracellular transport is generated by motor proteins, which bind to actin filaments or microtubules and use the energy derived from repeated cycles of ATP hydrolysis to travel along the actin filament or microtubule in a single direction. Motor proteins move along microtubules using their globular heads and they belong to two families:

1. The kinesins generally move toward the plus end of a microtubule; away from the centrosome
2. The dyneins move toward the minus end, or toward the centrosome

Kinesins and dyneins both have two globular ATP-binding heads and a tail. The globular heads are enzymes with ATP-hydrolyzing (ATPase) activity. This reaction provides the energy for a cycle of conformational changes in the head that enable it to move along the microtubule by a cycle of binding, release, and rebinding to the microtubule. Finally, microtubules and their associated motor proteins play an important part in positioning membrane-enclosed organelles within a eukaryotic cell.

As mentioned earlier in this summary, many microtubules in cells are stabilized through their association with other proteins, and therefore no longer show dynamic instability. This is seen in cilia and flagella that contain a bundle of stable microtubules. Bending of the microtubules, driven by a motor protein called ciliary dynein, causes their beating.

Actin filaments

Actin filaments are found in all eukaryotic cells and are essential for many of their movements, especially those involving the cell surface. Like microtubules, many actin filaments are unstable, but they can also form stable structures in cells, such as the contractile apparatus of muscle. The varied forms and functions of actin filaments in cells depends on multiple actin-binding proteins. These control the polymerization of actin filaments, cross-link the filaments into loose networks or stiff bundles, attach them to membranes, or move them relative to one another.

Composition of actin filaments

Actin filaments are helical polymers of actin molecules. They are thinner, more flexible, and shorter than microtubules. Furthermore, actin molecules rarely occur in isolation in the cell; they are generally found in cross-linked bundles and networks, which are much stronger then the individual filaments.

Actine filaments are polarized structures with a fast- and a slow-growing end; the rate of growth is faster at the plus end than at the minus end. A 'naked' actin filament is unstable and can disassemble from both ends. Each free actin monomer carriers a tightly bound ATP, which is hydrolyzed to ADP soon after the incorporation of the actin monomer into the filament. Hydrolyses of ATP to ADP reduces the strength of binding between monomers and decreases the stability of the polymer. This hydrolysis thereby promotes depolymerization, helping the cell to disassemble filaments after they have formed. As for microtubules, the ability to assemble and disassemble is required for many of the functions performed by actin filaments.

Drugs like cytochalasins prevent actin polymerization, and others like jasplakinolides stabilize actin filaments against depolymerization. Addition of these toxins freezes cell movements.

Function of actin filaments

Although actin is found throughout the cytoplasm of a eukaryotic cell, in most cells it is concentrated in the layer just beneath the plasma membrane, also known as the cell cortex. In this region actin filaments are linked by actin-binding proteins into a meshwork that supports the outer surface of the cell and gives it mechanical strength. In this way, actin filaments are responsible for the shape and movement of the cell surface, including the movements involved when a cell crawls along a surface. For this kind of movements are three interrelated processes required; all these three processes involve actin, but in different ways:

1. The cell pushes out protrusions at its front, or leading edge; this is driven by actin polymerization. The leading edge of a crawling cell regularly extends thin, sheetlike lamellipodia, which contain a dense meshwork of actin filaments. Many cells also extend thin, stiff protrusions called filopodia, which also contains a bundle of actin filaments. The formation and growth of actin filaments at the leading edge of a cell is assisted by various actin-binding accessory proteins, one set of proteins, the actin-related proteins or ARPs, promote the formation of branched actin filaments. These proteins form a complex that binds to the existing actin filaments, where they nucleate the growth of a new filament, which grows out at an angle to form a side branch. With the aid of additional actin-binding proteins, this web of actin undergoes assembly at the front end and disassembly at the back, pushing the lamellipodia to the front.
2. When the lamellipodia and filopodia touch down on a favorable patch of surface, the transmembrane proteins in their plasma membrane, known as integrins, adhere to molecules in the extracellular matrix on the surface of another cell over which the moving cell is crawling.
3. Meanwhile, on the intracellular face of the membrane of the crawling cell, integrins capture actin filaments, thereby creating an anchorage for the system of actin filaments inside the cell. The rest of the cell drags itself forward by traction on these anchorage points. This too depends on actin, but through the interaction of actin filaments with motor proteins known as myosins.

A variety of actin-binding proteins are thus required to drive the leading edge of a migrating cell forward, to adhere to the substratum, and to retract its trailing edge. All of these processes are triggered by external stimuli working via small GTP-binding proteins.

Muscle contraction

Myosins are motor proteins that use the energy of ATP hydrolysis to move along actin filaments: they can carry organelles along actin-filament tracks or cause adjacent actin filaments to slide past each other in contractile bundles. There are several different types of myosins in cells, of which the myosin-I and myosin-II subfamilies are most abundant. Myosin-I is found in all types of cells, and the myosin-I molecules have only one head

domain and a tail. The head domain interacts with actin filaments and has ATP-hydrolyzing motor activity; the tail varies among the different types of myosin-I.

Muscle myosin belongs to the myosin-II subfamily of myosins, all of which have two ATPase heads and a long, rodlike tail. Each myosin-II molecule is a dimmer composed of a pair of identical myosin molecules held together by their tails; it has two ATPase heads at one end and e single coiled-coil tail at the other. Clustering of myosin-II molecules bind to each other through their coiled-coil tails, forming a bipolor myosin filament in which the heads project from the side.

In muscle, huge arrays of overlapping actin filaments and myosin filaments generate contractions by sliding over one another. One set of heads binds to acting filaments in one orientation and moves them in one way; the other set of heads binds to other actin filaments in the opposite orientation and moves them in the opposite direction.

The long fibers of skeletal muscle are huge single cells formed by the fusion of many separate smaller cells. A skeletal muscle cell is packed with myofibrils, which consists of a chain of identical tiny contractile units, or sacromeres. Sacromeres are highly organized assemblies of two types of filaments: actin filaments and filaments of muscle-specific myosin-II. Myosin filaments are centrally positioned in each sacromere, whereas the actin filaments extend inward from each end of the sacromere and overlap the ends of the myosin filaments.
The contraction of a muscle cell is caused by simultaneous shortening of all the sacromeres, which in turn is caused by the actin filaments sliding past the myosin filaments, with no change in the length of either type of filament. When a muscle is stimulated to contract, the myosin heads start to walk along the actin filament in repeated cycles of attachment and detachment. During each cycle, a myosin head binds and hydrolyzes one molecule of ATP. After a contraction is completed, the myosin heads lose contact with the actin filaments completely, and the muscle relaxes.

The molecular interaction between myosin and actin filaments takes place only when the skeletal muscle receives a signal from the nervous system. The signal from a nerve terminal triggers an action potential, which caused a release of Ca2+ in the cytosol. As mentioned earlier in this summary, Ca2+ is widely used as an intracellular signal to rely a message from the exterior to the internal machinery of the cell. In the case of muscle, the Ca2+ interacts with a molecular switch made of specialized accessory proteins. One of these proteins is tropomyosin, a molecule that binds in the groove of the actin helix and prevents the myosin heads from associating with the actin filament. The other is troponin, a complex that includes a Ca2+-sensitive protein (troponin-C), which is associated with the end of a tropomyosin molecule. When the level of Ca2+ rises in the cytosol, Ca2+ binds to troponin and induces a change in its shape. This in turn causes the tropomyosin molecules to shift their position, allowing myosin heads to bind to the actin filament and initiating contraction.

18. Cell-cycle control and cell death

All living organisms are products of repeated rounds of cell growth and division extending back in time to the beginnings of life over three billion years ago. A cell reproduces by carrying out an orderly sequence of events in which it duplicates its contents and then divides in two. This cell cycle is the essential mechanism by which all living things reproduce. To ensure correct progression through the cell cycle, eukaryotic cells have evolved a complex network of regulatory proteins, known as the cell-cycle control system.

Overview of the cell cycle

The most basic function of the cell cycle is to duplicate accurately the vast amount of DNA in the chromosomes and then precisely distribute the copies into genetically identical daughter cells. The duration of the cell cycle varies greatly from one cell type to another.

The eukaryotic cell cycle consists of four distinct phases. In the M phase two events occur: dividing of the nucleus, a process called mitosis and dividing of the cytoplasm, a process called cytokinesis. The period

between one M phase and the next is called interphase, in which the cell increases in size and encompass the remaining three phases of the cell cycle. During S phase (S=synthesis), the cell replicates its nuclear DNA. S phase is flanked by two phases in which the cell continues to grow. The G1 phase (G=gap) is the interval between the completion of M phase and the beginning of the S phase. The G2 phase in the interval between the end of S phase and the beginning of M phase. During these gap phases, the cell monitors the internal and external environments to ensure that conditions are suitable and preparations are complete. At particular points in G1 and G2 the cell decides whether to proceed to the next phase or pause to allow more time to prepare.

The first visible sign that a cell is about to enter the M phase is the progressive condensation of its chromosomes, which were replicated earlier during S phase. Chromosome condensation thus marks the end of the G2 phase, and at this stage in the cell cycle the replicated chromosomes become visible in the light microscope.

The essential processes of the cell cycle, such as DNA replication, mitosis, and cytokinesis, are triggered by a cell-cycle control system. The events of the cell cycle must occur in a particular sequence, and this sequence must be preserved even if one of the steps takes longer than usual. The cell-cycle control system achieves all of this by means of molecular brakes that can stop the cycle at various checkpoints. Two important checkpoints occur in G1 and G2:

- The G1 checkpoint allows the cell to confirm that the environment is favorable for cell proliferation and its DNA is intact before committing to S phase. Cell proliferation depends of nutrients and specific signal molecules in the extracellular environment. If extracellular conditions are unfavorable cells can delay progress through G1 and may even enter a specialized resting state known as G0.
- The G2 checkpoint ensures that cells do not enter mitosis until damaged DNA is repaired and DNA replication is completed.

The cell-cycle control system

The cell-cycle control system coordinates the events of the cell cycle by cyclically switching on the appropriate parts of the cell-cycle machinery and then switching them off. As discussed earlier in this summary, phosphorylation followed by dephosphorylation is one of the most common ways used by cells to switch the activity of a protein on and then off, and the cell-cycle control system uses this mechanism repeatedly. The phosphorylation reactions are carried out by a specific set of protein kinases, enzymes that transfer a phosphate group from ATP to a particular amino acid side chain of the target protein. Switching these protein kinases on and off at the appropriate times is regulated by a second set of protein components of the control system: cyclin-dependent protein kinases (Cdks).

Different cyclin-Cdk complexes trigger different steps of the cell cycle:

- M-Cdk drives the cell into mitosis
- G1-Cdk drives it through G1 toward S phase
- G1/S-Cdk and S-Cdk drive it into S phase.

M-Cdk

Synthesis of M-cyclin starts immediately after cell division. Activated M-Cdk indirectly activates more M-Cdk; this explosive increase in M-Cdk activity drives the cell abruptly into M phase. Its rapid elimination then helps initiate the exit from mitosis. This elimination is the result of the ubiquitin-dependent proteolytic system. As mitosis nears completion, multiple molecules of the protein ubiquitin are attached to the M-cyclin molecules. This ubiquitination marks the cyclin for degradation in proteasomes, large proteolytic machines found in all eukaryotic cells. A protein complex called the anaphase promoting complex (APC) adds ubiquitin to the cyclin and to other proteins involved in the regulation of mitosis. Destruction of the cyclin inactivates the Cdk.

S-Cdk

As we discussed earlier, DNA replication begins at origins of replication. These sequences recruit specific proteins that control the initiation and completion of DNA replication. One multiprotein complex, the origin recognition complex (ORC), remains bound to the origins of replication throughout the cell cycle, where it serves as a sort landing pad for additional regulatory proteins that bind before the start of S phase. One of these regulatory proteins is called Cdc6, which concentration increases in early G1. When Cdc6 binds to ORCs in G1, it promotes the binding of additional proteins to form a pre-replicative complex. S-Cdk then triggers origin firing by causing the assembly of DNA polymerase and the initiation of DNA synthesis. S-Cdk also helps block replication by helping to phosphorylate Cdc6, which dissociated from the origin and is degraded.

The cell-cycle can be halted by at least two mechanisms:

1. 1Cdk inhibitor proteins can block the assembly or activity of one or more cyclin-Cdk complexes. This is seen when checkpoints mechanisms halts the cell cycle in G1 if DNA is damaged, helping to ensure that a cell does not replicated damaged DNA. When DNA is damaged, the p53 protein, which is normally rapidly degraded, is stabilized and activated. This is partly because p53 becomes phosphorylated by specific protein kinases that are activated in response to DNA damage. Activated p53 accumulates and stimulates the transcription of the gene that encodes the Cdk inhibitor protein p21. The p21 protein binds to G1/S-Cdk and S-Cdk and inactivates them, so that the cell cycle arrests in G1. If p53 is missing of defective, the replication of damaged DNA leads to a high rate of mutations and the production of cells that tend to become cancerous. In fact, mutations in the p53 gene are found in about half of all human cancers.
2. Components of the control system can stop being made, for example when cells enter G0. It seems to be a general rule that cells will multiply only if they are stimulated to do so by signals from other cells. If deprived of such signals, the cell cycle arrests at a G1 checkpoint and enters the G0 state.

Programmed cell death (apoptosis)

Animal cell numbers are not only regulated by controlling the rate of cell division, but also by controlling the rate of cell death. If cells are no longer needed, they commit suicide by activating an intracellular death program. This process is therefore called programmed cell death, although it is more commonly called apoptosis. In adult tissues, cell death exactly balances cell division. If this were not so, the tissue would grow or shrink.

Cells that die as a result of acute injury typically swell and burst, spilling their contents all over their neighbors, a process called cell necrosis. By contrast, a cell that undergoes apoptosis dies neatly, without damaging its neighbors. A cell in apoptosis shrinks and condenses. The cytoskeleton collapses, the nuclear envelope disassembles, and the nuclear DNA breaks up into fragments. Most important, the cell surface is altered in such a manner that it immediately attracts phagocytic cells, usually specialized phagocytic cells called macrophages. These cells engulf the apoptotic cell before it spills its contents. This rapid removal of the dying cell avoids the damaging consequences of cell necrosis, and also allows the organic components of the apoptotic cell to be recycled by the cell that ingests it.

Apoptosis is carried out by a family of proteases called caspases. The caspases are made as inactive precursors called procaspases, which are themselves activated by proteolytic cleavage in response to signals that induce apoptosis. The activated caspases cleave, and thereby activate, other members of the family, resulting in an amplifying proteolytic cascade.

The main proteins that regulate the activation of procaspases are members of the Bcl-2 family of intracellular proteins. Some members of this protein family promote procaspase activation and cell death, whereas others inhibit these processes. Two of the most important death-promoting family members are proteins called Bax and Bak. These proteins activate procaspases indirectly, by inducing the release of cytochrome c from mitochondria into the cytosol. Cytochrome c binds to an adaptor protein, which then activates a specific procaspase. This activated procaspases initiates the caspase cascade that leads to apoptosis.

Extracellular control of cell numbers and cell size

Most of the extracellular signal molecules that influence cell division, cell growth, and cell survival are soluble proteins secreted by other cells or proteins bound to the surface of other cells or the extracellular matrix. Although most act positively to stimulate one or more of these cell processes, some act negatively to inhibit a particular process. The positively acting signal proteins can be divided into three major classes based on their function:

1. *Mitogens*: animal cells proliferate only if stimulated by mitogens, which activate intracellular signaling pathways to override the normal brakes that otherwise block cell-cycle progression; this mechanism ensures that a cell divides only when another cell is needed.
2. *Growth factors*: for an organism or an organ to grow, cells must grow as well as divide. Animal cell growth depends on extracellular growth factors, which stimulate protein synthesis and inhibit protein degradation.
3. *Survival factors*: most animal cells require continuous signaling from other cells to avoid apoptosis; and this may be a mechanism to ensure that cells survive only when and where they are needed.

Cancer cells fail to obey these normal 'social' controls on cell behavior and therefore outgrow, out-divide and out-survive their normal neighbors.

19. Cell division

We take a closer look to the final phase of the cell cycle, when the cell divides its nucleus (mitosis) and then its cytoplasm (cytokinesis). Together, mitosis and cytokinesis constitute M phase of the cell cycle.

An overview of M Phase

When the chromosomes are duplicated in S phase, the two copies of each duplicated chromosome (called sister chromatids) remain tightly bound together by cohesins. A set of protein complexes, called condensins, helps to carry out chromosome condensation when the cell is about to enter the M phase. Together, cohesins and condensins help reduce the mitotic chromosomes to small, condensed structures that can be easily segregated during mitosis. M phase is initiated by the phosphorylation triggered by activated M-Cdk.

To produce two identical daughter cells, a mitotic spindle is formed during M phase. This mitotic spindle is composed of microtubules and the various proteins that interact with them, including microtubule-associated motor proteins. In animal cells and many unicellular eucaryotes, a different cytoskeletal structure is responsible for cytokinesis. It is called the contractile ring because it consists mainly of actin filaments and myosin filaments arranged in a ring around the equator of the cell. As the ring contracts, it pulls the membrane inward, thereby dividing the cell in two. The microtubules bind to protein complexes called kinetochores, associated with the centromere of each sister chromatid.

However, before M phase begins two critical events must be completed: DNA must be fully replicated and the centrosome must be duplicated. Each centrosome consists of proteins that contain hundreds of g-tubulin rings. These ring complexes serve as nucleation sites for the growth of microtubules that radiate out from the centrosome. During interphase of each cell cycle, the centrosome is duplicated and as mitosis begins the two centrosomes separate, each of which nucleates its own aster. The two asters move to opposite sides of the nucleus to form the two poles of the mitotic spindle. When the nuclear envelope breaks down, the spindle microtubules invade the nuclear area and capture the replicated chromosomes. The process of centrosome duplication and separation is known as the centrosome cycle.

Although M phase proceeds as a continuous sequence of events, it is traditionally divided into six stages. The first five stages of M phase constitute mitosis, and cytokinesis occurs in the sixth stage, which overlaps with the end of mitosis.
The six stages are:

1. *Prophase:* at the beginning of the prophase, the two daughter centrosomes separate. They now organize their own array of microtubules and being to move to opposite poles of the cell, driven by centrosome-associated motor proteins that use the energy of ATP hydrolysis to move along the microtubules. During prophase, some of the microtubules growing from one centrosome interact with the microtubules from the other centrosome; this interaction stabilizes the microtubules.
2. *Prometaphase:* this starts abruptly with the disassembly of the nuclear envelope, which breaks up into small membrane vesicles. This process is triggered by the phosphorylation and consequent disassembly of the intermediate filament proteins of the nuclear lamina. Chromosomes can now attach to spindle microtubules via their kinetochores and undergo active movements.
3. *Metaphase:* the chromosomes are aligned at the equator of the spindle, midway between the spindle poles. The paired kinetochore microtubules on each chromosome attach to opposite poles of the spindle.
4. *Anaphase:* this begins abruptly with the release of the cohesin linkage that holds the sister chromatids together. This allows each chromatid (now called a daughter chromosome) to be gradually pulled to the spindle pole to which it is attached. This movement segregates the two identical sets of chromosomes to opposite ends of the spindle. The abrupt disruption of the cohesin linkage is triggered by the activation of the anaphase-promoting complex (APC). Once this proteolytic complex is activated, it cleaves an inhibitory protein, thereby releasing a proteolytic enzyme that breaks the cohesin linkage. The anaphase is separated in two processes: in anaphase A the kinetochore microtubules shorten by depolymerization, and the attached chromosomes move poleward. In anaphase B, the spindle poles themselves move apart, further contributing to the segregation of the two sets of daughter chromosomes.
5. *Telophase*: the two sets of daughter chromosomes arrive at the poles of the spindle. A new nuclear envelope reassembles around each set, completing the formation of two nuclei and marking the end of mitosis. The division of the cytoplasm begins with the assembly of the contractile ring.
6. *Cytokinesis*: in animal cells, cytoplasmatic division is mediated by a contractile ring of actin filaments and myosin filaments, which assembles midway between the spindle poles and contracts to divide the cytoplasm in two; in plant cells, by contrast, cell division occurs by the formation of a new cell wall inside the cell, which divides the cytoplasm in two.

Together, the six stages form a dynamic sequence in which many independent cycles are coordinated to produce two genetically identical daughter cells.

The process of mitosis ensures that each daughter cell receives a full complement of chromosomes. But when a eucaryotic cell divides, each daughter cell must also inherit all of the other essential cell components, including the membrane-enclosed organelles. Therefore, large membrane-enclosed organelles such as the endoplasmatic reticulum and Golgi apparatus break into many smaller fragments during M phase. Other components of the cell, including all of the soluble proteins, are inherited randomly when the cell divides its cytoplasm in the final stage of the M phase.

20. Genetics, meiosis, and the molecular basis of heredity

The benefits of sex

Most of the organisms around us reproduce sexually. However, reproduction without sex is possible; bacteria and other single-celled organisms can reproduce by simple cell division. While asexual reproduction is simple en direct, it usually gives rise to offspring that are genetically identical to the parent organisms. Sexual reproduction, on the other hand, involves the mixing of genomes from two individuals to produce offspring that are genetically distinct from one another and from both their parents. This way of reproduction has great advantages. One advantage seems to be that the reshuffling of the genes through sexual reproduction can help a species survive in an unpredictably variable environment. If two parents produce many offspring with a wide variety of gene combinations, the chance that at least one of their progeny will have the combinations of features necessary for survival is increased.

Sexual reproduction occurs in diploid organisms, in which each cell contains two sets of chromosomes, one inherited from each parent. The specialized cells that carry out sexual reproduction (the germ cells, or gametes) are haploid: they each contain only one set of chromosomes. These haploid germ cells are generated when a diploid cell undergoes the process of cell division called meiosis. During meiosis the chromosomes of the double chromosome set are portioned out into single chromosome sets. The two different haploid gametes then fuse to make a diploid cell (the zygote) with a new combination of chromosomes. The zyg ote thus produces develops into a new individual with a diploid set of chromosomes, which differs from that of either parent. In this way, a distinction can be drawn between the cells of the germ line (from which the next generation of gametes will be derived) and the somatic cells (which form the rest of the body).
Sexual reproduction thus involves the cyclic alternation of diploid and haploid states: diploid cells divide by meiosis to form haploid gametes, and the haploid gametes from two individuals fuse as fertilization to form a new diploid cell.

Meiosis

During meiosis, the maternal and paternal chromosomes of a diploid cell are parceled out to gametes so that each gamete receives one copy of each chromosome. Because the assortment of the two members of each chromosome pair occurs at random, many genetically different gametes can be produced from a single individual.

Before a cell divides – by either meiosis or mitosis – it first duplicates all of its chromosomes. The twin copies of each fully replicated chromosome at first remain tightly linked along their length and are called sister chromatids. However, the way these replicated chromosomes are handled differs in meiosis and mitosis. In mitosis the replicated chromosomes line up at random order at the metaphase plate; as mitosis continues, the two previously joined sister chromatids then separate from each other to become individual chromosomes. The events that occur in the first meiotic cell division mirror the sequence of stages that a cell goes through in mitosis: in prophase, the replicated chromosomes condense; in metaphase, they align at the equator of the meiotic spindle; and in anaphase, they are segregated to the poles. In this first meiotic division the homologous maternal and paternal chromosomes have paired before lining up at the metaphase plate.

After the duplicated homologs pair, genetic recombination is initiated, also called crossing-over. Crossing-over ensures the proper segregation of homologous chromosomes and enhances the genetic reassortment that occurs during meiosis by exchanging genes between them. The structure formed when homologous chromosomes pair is called a bivalent and consists of four chromatids. Cross-over events between a maternal and paternal chromatid in paired chromosomes forms a chiasma (a connection that corresponds to a crossover between two non-sister chromatids). The combination of the chiasmata and the tight attachment of the sister chromatids to each other mediated by cohesin proteins, holds the two duplicated homologs together until the spindle separates then at anaphase I. In most organisms, recombination during meiosis is required for the correct segregation of the two duplicated homologs into separate daughter nuclei.

The first meiotic division does not produce cells with a haploid amount of DNA. To achieve this goal, each cell proceeds through a second round of division, meiosis II, which occurs without further DNA replication and without any significant interphase period. A spindle forms, the chromosomes align at its equator, and the sister chromatids separate to produce daughter cells with a haploid DNA content. In meiosis II, as in mitosis, the kinetochores on each sister chromatid function independently and the cohesins holding the sister chromatids together at the centromere are degraded, allowing the two sister chromatids to be pulled to opposite poles.

Although most of the mechanical features of meiosis are similar to those of mitosis, the behavior of the chromosomes is different: meiosis produces four genetically dissimilar haploid cells by two consecutive cell divisions, whereas mitosis produces two genetically identical diploid cells by a single cell division. Whereas mitosis and division II of meiosis usually occur within hours, division I of meiosis can last days, month, or even years, due to the long time spent in prophase.

Occasionally, homologs fail to separate properly, a phenomenon known as nondisjunction. As a result, some of the haploid cells that are produced lack a particular chromosome, while others have more then one copy of it. Upon fertilization, such gametes form abnormal embryos, most of which die. However, some survive: Down syndrome, caused by an extra copy of Chromosome 21, is a human disease characterized by severe mental retardation. This extra copy results from nondisjunction of a Chromosome 21 pair during meiosis, giving rise to a gamete that contains two copies of Chromosome 21 instead of one copy. When this abnormal gamete fuses with a normal gamete at fertilization, the resulting embryo contains three copies of Chromosome 21 instead of two.
Regardless of whether the segregation error occurs in the sperm or the egg, nondisjunction is thought to be one of the reasons for the high rate of miscarriages (spontaneous abortion) in early pregnancy in humans.

Mendel and the laws of inheritance

Mendel unraveled the laws of heredity by studying the inheritance of a handful of discrete traits in garden peas. He supposed that for any pair of alleles, one allele is dominant and the other is recessive. The ideas of homozygosity and heterozygosity are also his. One important consequence of heterozygosity, and of dominance and recessiveness, is that not all of the alleles an individual carries can be detected in its phenotype. Humans have about 30.000 genes, and each of us is heterozygous for a very large number of these. Thus, we all carry a great deal of genetic information that remains hidden in our personal phenotype, but that can turn up in future generations.

Mendel's first law

The law of segregation states that the maternal and paternal alleles for each trait separate from one another during gamete formation, then reunite at random during fertilization. This law permits us to predict the phenotypes that will result from a particular cross-breeding experiment. Mendel's law of segregation also explains the 3:1 ratio that can be observed in the F2 generation of heterozygous inheritance. But his rules governing inheritance are not limited to reproduction in plants. Mendel's concept of the gene and his law of segregation can be generalized to all sexually reproducing organisms, including humans.

Mendel's second law

The law of independent assortment states that during gamete formation, different alleles segregate independently of each other. Furthermore, the behavior of chromosomes during meiosis explains Mendel's laws. During meiosis, each set of paired homologs attaches to the spindle independently. This random arrangement of chromosomes on the metaphase spindle is reflected in Mendel's law of independent assortment, since gene on different chromosomes will be inherited independently.

Mendel's observation that different genes assort independently does not necessarily require that the genes lie on different chromosomes. Genes that are far enough away from one another on the same chromosome will also sort independently due to the recombination that occurs during prophase of meiosis I. In this way, if genes lie close together on a chromosome they tend to be inherited as a unit. By measuring how frequently genes are co-inherited, researchers can determine whether genes reside on the same chromosome and, if so, how far apart they lie. This type of information has been used to map the relative positions of the genes on each chromosome of many organisms. Such genetic maps have been crucial for cloning of human disease genes such as the gene for cystic fibrosis.

Gene mutations

Mutant alleles can be either dominant or recessive. If the heterozygous organism has a mutant phenotype, the mutant allel is dominant; if it has a normal phenotype, the mutant allel is recessive. So, the key to determining whether a particular allele is dominant or recessive lies in the phenotype of the heterozygote.

Mutations have very different consequences, some confer a selective advantage, whether others cause defects and make the organisms less likely to survive. However, even deleterious mutations can benefit an organism, as individual genes can have multiple effects on phenotype. Take sickle-cell anemia in humans: this disorder is caused by a mutation in the gene encoding for b-globin, one of the polypeptides that make up hemoglobin. The sickle-cell mutation directs the formation of an abnormal polypeptide that causes red blood cells to adopt to a sickled shape. These misshapen cells clog small blood vessels, reducing the amount of oxygen that can reach different tissues, causing a variety of symptoms including muscle cramps and even heart failure. But the sickle-trait also has its benefits! Individuals who are heterozygous or homozygous for the sickle-mutation, are resistant to malaria. This is because the organism that causes the disease is unable to reproduce in sickle-shaped red blood cells, which fragment before the parasite has a chance to multiply.

Genetics as an experimental tool

Before the advent of recombinant DNA technology, most genes were identified by observing he processes disrupted when the gene was mutated. Although spontaneous mutations can sometimes be found by examining extremely large populations the process of identifying interesting mutants can be made much more efficient by generating mutations with agents that damage DNA, called mutagens. Different mutagens can generate different types of DNA alterations. And such mutants can then be screened to identify phenotypes of interest and, ultimately, to isolate the responsible genes.

Unlike worm and flies, humans are not suitable for experiments with mutagens, because they do not reproduce rapidly and any human with a serious defect in an essential process, such as DNA replication, would die long before birth. We study human genes, by studying less complex organisms which genes can reveal critical information about similar genes and processes in humans. And secondly, by analyses of the phenotypes of individuals with spontaneous mutations (like sickle-cell anemia), together with studies of their cultured cells, have provided many unique insights into important human gene function.

A large-scale genetic screen can turn up many different mutants that have the same phenotype. These defects might lie in different genes that function in the same process, or they might represent different mutations in the same gene. Complementation tests can be used to ascertain whether the mutations fall in the same or in different genes. In the simplest type of complementation test, an individual that is homozygous for one mutation is mated with an individual that is homozygous for the other mutation. If the two mutations are in the same gene, the offspring will show the mutant phenotype, because they carry only defective copies of the gene in question. If, in contrast, the mutations fall in different genes, the resulting offspring will show ‘the normal phenotype’ because they will have one normal copy (and one mutant copy) of each gene. The mutations thereby complement one another and restore a normal phenotype.

With the exception of identical twins, no two humans genomes are alike. Each of us carries a unique set of polymorphisms – changes in nucleotide sequence – that shapes our individual phenotypes. Single-nucleotide polymorphisms (SNPs) are DNA sequences that differ by a single nucleotide base between one portion of the population and another. They provide useful markers for performing genetic analyses that link a specific trait with a particular region of DNA. These polymorphisms can be used as markers for building genetic maps or for performing the genetic analyses that allow us to link particular polymorphisms with specific disease or predispositions to disease.

The problem is that any two humans differ about 0.1% in their nucleotide sequences. Theoretically, one would need to search through all 3 million of those polymorphisms to identify the one or two changes that are responsible for the differences. However, to reduce the number of polymorphisms one needs to examine, researchers are taking advantage of the recent discovery that human genes tend to be inherited in blocks. These haplotype blocks contain sets of alleles and SNPs that have been inherited as a group with little genetic rearrangement. The presence of such genetic clusters makes it easier to identify genes and mutations that are associated with human disease.

Many human traits run in families, have a genetically inherited component, but do not adhere strictly to Mendel’s laws. These complex traits are often polygenic; they arise from the interactions of multiple genes,

each of which makes a small contribution to the phenotype. Although many human traits have a strong genetic basis, some are determined primarily by the environment. It is the interactions of our genetic makeup with our environment that make each of us unique.

21. Tissue and cancer

Cells are the building blocks of multicellular organisms. Most of the cells are organized into cooperative assemblies called tissues. However, tissues are composed not only of cells, with their internal framework of cytoskeletal filaments and extracellular matrix. It is this matrix that gives supportive tissues their strength.

Plants and animals have evolved their multicellular organization independently, and their tissues are constructed on different principles. Because the cytoskeleton of plants lacks the tension-bearing intermediate filaments found in animal cells, and it has virtually no tensile strength. An external cell wall, therefore, is essential. Animal tissues are more diverse. Like plants, they consists of extracellular matrix as well as cells, but these components are organized in many different ways, including bone, muscle, and epidermis.

Extracellular matrix and connective tissues

Plants

In plants, each cell surrounds itself with extracellular matrix in the form of a cell wall. Most newly formed cells in a multicellular plant initially make relatively then primary cell walls that are capable of slowly expanding to accommodate subsequent cell growth. The driving force for growth is a swelling pressure, called the turgor pressure, which develops due to an osmotic imbalance between the interior of the cell and its surroundings. Once growth stops and the wall no longer need to expand, a more rigid secondary cell wall is produced. Cellulose fibers give the plant cell wall its tensile strength. In woody tissue, a highly cross-linked lignin network is deposited within this matrix to make it more rigid and waterproof.

Because the cellulose fibers resist stretching, their orientation governs the direction in which the growing cell enlarges, or grows. Cellulose is synthesized on the outer surface of the cell by enzyme complexes embedded in the plasma membrane. These transport sugar monomers across the membrane and incorporate them into a set of growing polymer chains at their points of membrane attachment. Each set of chains forms a cellulose microfibril. The enzyme complexes move in the membrane, spinning out new polymers and laying down a trail of oriented cellulose fibers behind them. Just underneath the plasma membrane, microtubules are aligned exactly with the cellulose fiber outside the cell. These microtubules are thought to serve as tracks to guides the movement of the enzyme complexes. In this way, the orientation of the cytoskeleton controls shaping of the plant cell and the modeling of the plant tissue.

Cellulose fibers in the plant cell wall confer tensile strength; other cell wall components give resistance to compression.

Animals

There are four major types of tissues in animals: connective, epithelial, nervous, and muscular. But the basic distinction is between connective tissue and the rest. Animal connective tissues provide mechanical support and consist of extracellular matrix with sparsely scattered cells. In the extracellular matrix of animals, the tensile strength is provided not by a polysaccharide, as in plants, but by a fibrous protein called collagen. The various types of connective tissue owe their specific characters to the type of collagen that they contain, to its quantity, and to the other molecules that are interwoven it in varying proportions. The protein and polysaccharide components of the matrix are made by connective tissue cells embedded in it; in most connective tissues these cells are called fibroblasts; in bone they are called osteoblasts.

Some people have a genetic defect in the collagenase, so that their collagen fibrils do not assemble correctly. As a result, the skin and various other connective tissues have reduced tensile strength and are extraordinarily stretchable.

Cells must be able to attach to the matrix, but cells do not attach well to bare collagen. Another extracellular matrix protein, fibronectin, provides a linkage; one part of the fibronectin molecule binds to collagen, while another par forms an attachment site for a cell. The cell binds to the specific site by means of a receptor protein, called an integrin. While the extracellular domain of the integrin binds to fibronectine, the intracellular domain binds to actin filaments.

While collagen provides tensile strength to resist stretching; glycosaminoglycans (GAGs), covalenty linked to proteins to form proteoglycans, act as space-filters and provide resistance to compression.

Epithelial sheets and cell-cell junction

Cells joined together in epithelial sheets line all external and internal surfaces of the animal body. An epithelial sheet has two faces: the apical surface is free and exposed to the air or to a watery fluid; the basal surface rests on some other tissue to which it is attached. Supporting the basal surface of the epithelium there lies a thin tough sheet of extracellular matrix, called the basal lamina, composed of a specialized type of collagen and various other molecules, including a protein called laminin. Laminin provides adhesive site for integrin molecules in the plasma membrane of the epithelial cells. The apical and basal faces of an epithelium are chemically different, reflecting a polarized internal organization of the individual epithelial cells.

In epithelial sheets, in contrast to connective tissue, tension is transmitted directly from cell to cell via cell-cell junctions. Several types of cell-cell junctions are found in epithelia in animals:

- Tight junction
- Adherens junction
- Desmosome
- Gap junction
- Hemidesmosome

Tight junctions seal neighboring cells together in an epithelial sheet to prevent leakage of molecules between them. The tight junctions are formed from proteins called claudins and occludins, which are arranged in strands along the lines of junction to create the seal. As we saw earlier in this summary, tight junctions also play a key part in maintaining the polarity of the individual epithelial cells.

The junctions that hold an epithelium together by forming mechanical attachments are of three main types. Adherens junctions and desmosome junctions bind one epithelial cell to another, while hemidesmosomes bind epithelial cells to the basal lamina. Adherens junctions and desmosome junctions are both build around transmembrane proteins belonging to the same family, called cadherins. A cadherin molecule in the plasma membrane of one cell binds directly to an identical cadherin molecule in the plasma membrane of its neighbor, this kind of binding is called homophilic.

Proteins of the cadherin family span the epithelial cell membrane and bind to similar cadherins in the adjacent epithelial cell. At an adherens junction, the cadherins are linked intercellularly to actin filaments; at a desmosome junction, they are linked to keratin filaments. Actin bundles connected from cell to cell across an epithelium can contract, causing the epithelium to bend.
Gap junctions form channels that allow passage of small molecules and ions from cell to cell. Curiously, plant tissues, though they lack all the other types of cell-cell junctions, have a functional counterpart of the gap junction. The cytoplasms of adjacent plant cells are connected via minute communicating chancels called plasmodesmata, which span the intervening cell walls.

Tissue maintenance and renewal

Although the final structure of an animal's body may be enormously complex, it is generated by a limited repertoire of cell activities. Examples of all these activities have been discussed earlier in this summary. Trough cell division, cell growth, cell movement, and cell specialization, a fertilized egg cell gives rise to a multicellular animal. Almost every tissue in animals consists of a complex mixture of cell types that are subject to continual turnover. But throughout cell replacement and tissue renewal, the organization of the tissue must be preserved. Three key factors maintain the cellular organization of tissues:

1. *Cell communication:* to survive most cells have to receive signals from their environment. These communications ensure that new cells are produced and survive only when and where they are required
2. *Selective cell-cell adhesion:* because different cell types have different cadherins and other adhesion molecules in their plasma membranes, they tend to stick selectively (by homophilic binding) to other cells of the same type. Sometimes they form selective attachment to certain other cell types or to specific extracellular matrix components. The selectivity of adhesion prevents the different cell types in a tissue from becoming chaotically mixed
3. *Cell memory:* specialized patterns of gene expression, evoked by signals that acted during embryonic development, are maintained so that cells preserve their distinctive character and pass it on to their progency

Cells in tissue vary enormously in their rate and pattern of turnover. At one extreme are nerve cells, most of which last a lifetime without replacement. At the other extreme are the cells that line the intestine, which are replaced every few days. Between these extreme there is of course a spectrum of different rates and styles of cell replacement and tissue renewal. Our life depends on these renewal processes. A large dose of ionizing radiation, by blocking cell division, halts renewal.

Many of the differentiated cells that need continual replacement are themselves unable to divide. Red blood cells, surface epidermal cells, and the absorptive and goblet cells of the gut lining are all of this type; also referred as terminally differentiated cells. Replacement for terminally differentiated cells are generated from a stock of proliferating precursor cells, which themselves usually derive from small numbers of more slowly dividing stem cells. The stem cells and proliferating precursors cells are retained in the corresponding tissues along with the differentiated cells. When a stem cell divides, each daughter can either remain a stem cell or go on to become terminally differentiated. The pattern of replacement varies from one stem-cell-based tissue to another. Often, a single type of stem cell gives rise to several types of differentiated progency; the process of blood-cell formation, or hemopoiesis, provides an extreme example of the phenomenon. All of the different cell types in the blood ultimately derive from the same hemopoietic stem cell that normally inhabits the bone marrow.

Embryonic stem cells (ES cells) can be maintained indefinitely in culture and remain capable of differentiating into any cell type in the body. Perhaps one day it may even become possible to grow entire organs from ES cells by recapitulation of embryonic development. There is, however, one major problem associated with the use of ES cells for tissue repair. If the transplanted cells are genetically different from the cells of the patient into whom they are grafted, they are likely to be rejected and destroyed by the immune system.
By nuclear transplantation, personalized ES cells can in principle be produced for any adult, a technique called 'therapeutic cloning'. In therapeutic cloning only cells are produced; in reproductive cloning a whole new multicellular individual is generated.

Cancer

Foremost among the diseases of tissue renewal is cancer. In Europe and North America, one in four of us will die of cancer. Cancer cells fail to obey the social constraints that normally maintain tissue organization: they proliferate when they should not, survive where they should not, and invade regions that they should keep out of. It is the combination of these features that creates the lethal danger.

Epidemiology has provided strong evidence that the environment plays a part in the causation of most cases of cancer. Although it is still hard to discover which specific factors in the environment or life-style are critical, and many remain unknown, some of them have been identified quite precisely. Obesity, for example, is

correlated with an increased cancer risk, and the relationship is suspected to be causal. By far the most important environmental cause of cancer, however, is tobacco-smoking, which is not only responsible for almost all cases of lung cancer, but also raises the incidence of several other cancers, such as those of the bladder.

Cancer is fundamentally a genetic disease: it arises as a consequence of pathological changes in the information carried by DNA. It differs from other genetic diseases in that the mutations underlying cancer are mainly somatic mutations as opposed to germ-line mutations. Most of the identified agents known to contribute to the causation of cancer are mutagens: they cause changes in the nucleotide sequence of DNA. But mutations can also occur spontaneously as a result of fundamental limitations on the accuracy of DNA replication and DNA repair. Nevertheless, it takes more than a single mutation to turn a normal cell into a cancer cell. In fact, cancer cells arise from the accumulation of many mutations in a single somatic cell lineage. And cancer, therefore, is typically a disease of old age.
To be successful, a cancer cell must acquire a whole range of abnormal properties as it evolves. Different cancers require different combinations of properties. Even though, there is a general list of key behaviors of cancer cells that distinguish them from normal cells:

1. They have a reduced dependence on signals from other cells for their growth, survival, and division. A mutation in a ras gene can, for example, cause an intercellular signal for proliferation to be produced even in the absence of the extracellular signal that would normally be needed to trigger it.
2. Cancer cells are less prone than normal cells to kill themselves by apoptosis. This is often caused by mutations in genes that regulate the intracellular death program, including the p53 protein. Mutations in the p53 gene, allowing them to survive and divide even when their DNA is damaged.
3. Unlike most normal human cells, cancer cells can often proliferate indefinitely by reactivating production of the telomerase enzyme that maintains telomere length.
4. Most cancer cells are genetically unstable, with a greatly increased mutation rate.
5. Cancer cells are abnormally invasive, and this is often in part because they lack specific cell-adhesion molecules, such as cadherins, that hold normal cells in their proper place.
6. Cancer cells can often survive and proliferate in foreign tissues to form metastases, whereas most normal cells die when misplaced.

Many diverse types of genes are critical for cancer. In some case, the dangerous mutations are ones that make the affected gene product hyperactive. These mutations have a dominant effect and the mutant gene is called an oncogene; the corresponding normal form of the gene is then called a proto-oncogene. For other genes, the danger lies in mutations that destroy gene function. These mutations are generally recessive and the affected gene is called a tumor suppressor gene. Tumor suppressor genes can sometimes be identified through studies of rare cancer-prone families in which a mutation of one gene copy is inherited.

Colorectal cancer illustrates how loss of a gene can lead to growth of a tumor. Colorectal cancer arises from the epithelium lining the colon and rectum; most cases are seen in old people and do not have any discernible hereditary cause. A small proportion of cases, however, occur in families that are exceptionally prone to the disease and show an early onset. In one set of families, the predisposition to cancer has been traced to an inherited mutation in a DNA repair enzyme. In another class of hereditary colorectal cancer patients, a different mutation is present, leading to a highly distinctive phenotype. The affected individuals develop colorectal cancer in early adult life, and the onset of their disease is foreshadowed by the development of hundreds of thousands of little tumorous growths, called polyps, in the lining of the colon and rectum. The abnormality can be traced to deletion or inactivation of a gene called the adenomatous polyposis coli (APC) gene. Affected individuals inherit one mutant copy of the gene and one normal copy; their cancers arise from cells that can be shown to have undergone a somatic mutation that inactivates the remaining good copy. All this identifies APC as a tumor suppressor gene. When APC is lost, the ‘Wnt pathway’ (which is involved in stimulating cell proliferation in crypts of the gut lining) is hyperactive and the cells proliferate to excess, generating a polyp. Within this growing mass of tissue, further mutations may occur, resulting in invasive cancer.

An understanding of cancer cell biology opens the way to new treatments. At this moment, surgery remains the most effective tactic in many cancer, and surgical techniques are continually improving. Where surgery

fails, therapies based on the intrinsic peculiarities of cancer cells can be used. One promising strategy for the future is to block formation of the new blood vessels that normally invade a growing tumor, and so to choke tumor growth by depriving the cells of their blood supply. Another strategy aims to use antibodies that bind to the tumor-specific cell surface proteins; the antibodies can be coupled to toxins or toxin-generating enzymes that will kill the targeted cancer cells.

With our modern understanding of the molecular biology of cancer, we hoop it will be possible to dives effective methods of treatment for a still wider range of forms of cancer.