

Overview of Cell Biology

The Cell

- Cells are the fundamental unit of life; the **structural and functional unit** of all living organisms.
- The cell theory: All organisms are composed of cells and all cells come from pre-existing cells.
- In single-celled organisms such as bacteria and protozoa, each cell is independent.
- In multi-celled organisms, function is distributed among different specialized cells.
- Cells to tissues to organs to organisms...
- Biological systems use cells to build higher levels of organization, but the cell remains the fundamental unit. Radiation effects at the cellular level can affect the higher-level organization.

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[Lodish, 2000]

What can cells do?

Cells can:

- use DNA as hereditary material,
- use proteins as catalysts
- reproduce
- transform matter and energy
- respond to their environment

Sizes and shapes vary

0.5 μm bacteria to a several cm hen egg (yolk)

shapes can be spherical, flattened columnar, cuboidal.

The cell membrane

- The interior of cells is an aqueous environment.
- Most of the intracellular molecules are water-soluble.
- Most of the chemical reactions carried out inside cells require an aqueous environment.

- The environment around cells is also basically an aqueous one.
- Bodily fluids and blood are aqueous solutions of proteins and small molecules.
- Chemically (pH, ionic strength) blood is similar to sea water.

For cells to maintain integrity they must be surrounded by a membrane through which water cannot flow. A membrane composed of fatty acid molecules served this purpose.

All cells have a **cell membrane**, a two-layered shell of phospholipids. All biological membranes have the same basic phospholipid bilayer structure.

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Other functions of membranes

Membranes serve many functions other than segregating inside from outside. Many proteins are associated with membranes, either embedded in the membrane, or attached to the surface.

- **Transport** of ions or macromolecules
- **Energy storage.** Ion concentration gradients serve as source of potential energy.
- **Receptors** for signal transduction
- **Adherence.** Cell-cell contacts.

Two major divisions of cells.

Prokaryotes (before nucleus). Lack a defined nucleus. Have a simplified internal organization. Have only a plasma membrane and a nucleoid, no internal membranes.

All are single celled organisms (e.g., bacteria, blue-green algae)

Eukaryotes (typical or true nucleus). All members of the plant and animal kingdoms are eukaryotes. Eukaryotes have a more complicated internal structure including a defined, membrane-limited nucleus, and several distinct, membrane-delimited **organelles**.

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[Lodish, 2000]

Two major divisions within the cell.

Nuclear region (information storage and processing)

Cytoplasm (diverse functions, organelles)

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[from Lodish, 2000]

Some definitions:

Plasma membrane: surrounds the outside of the cell, 7-8 nm thick, responsible for transport, receptors (signal transduction) adherence, immune recognition.

Nuclear envelope: separates the nucleus and the cytoplasm; a **double membrane** with nuclear pores (channels for conducting materials between the nucleus and the cytoplasm)

Nucleus. Contains **DNA** associated with proteins called histones and non-histone chromosomal proteins.

Histones- small positively charged proteins mainly structural, that pack DNA into chromatin fibers.

Nonhistone proteins- involved in DNA replication, transcription regulation of gene activity

Chromosome: each individual linear DNA molecule with its associated proteins;

23 pairs of chromosomes in humans (ranges from 1 to 50)
the same in all cells of an organism

only visible during mitosis
in humans each chromosome contains about a meter of DNA

Chromatin: DNA and its associated proteins; the collection of chromosomes at any phase of the cell cycle.

Gene: the information encoded in the DNA sequence necessary to make a protein

Genome: all of the DNA, or hereditary material, in both functional and nonfunctional sequences in an organism.

Cytoplasm: the region of the cell lying outside the nucleus.

Ribosomes: site of translation (protein synthesis); may be free in cytoplasm or attached to endoplasmic reticulum.

Rough endoplasmic reticulum: ER with attached ribosomes; proteins formed here go into ER to be further processed and/or distributed to vesicles for transport to other parts of the cell.

Smooth ER: no ribosomes attached; functions include breaking down fats and synthesis of lipids.

Golgi apparatus: direct membrane constituents to appropriate locations within the cell; function in the formation of storage vesicles or secretory vesicles.

Lysosomes: organelles in animal cells that containing digestive enzymes.

Mitochondria: carry out the cell’s energy metabolism is carried out. Mitochondria contain DNA. Function?

Peroxisomes: fatty acids and amino acids are degraded

Cytoskeleton: supportive network in the cytosol composed of three types of fibers:

- **Microtubules:** fibers built of polymers of tubulin; responsible for cell movement,
- **Microfilaments,** built of the protein actin
- **intermediate filaments.**

The cytoskeleton gives the cell strength and rigidity control movement of structures within the cell, provide tracks along which organelles move.

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[Lodish, 2000]

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Biomolecules:

Cells carry out a multitude of chemical transformations, which provide energy, and the molecules needed to form its structure and coordinate its activities.

Small molecules: Water, inorganic ions, and a small array of small organic molecules (e.g., sugars, vitamins, fatty acids), comprise 75-80% of living matter by weight. These are **imported into cells**. Cells can also make or transform many small molecules as part of the cells metabolic activity.

Macromolecules: proteins, polysaccharides, phospholipids and DNA. These are **made within cells**.

- **Carbohydrates:** carbon hydrogen and oxygen (ratio 1:2:1); sugars (monosaccharides) usually 3-7 carbons, straight chains or rings, found in starches, cellulose, glycoproteins
- **Lipids:** diverse group of compounds that dissolve more readily in nonpolar solvents than in water
 - Fatty acids
 - Saturated
 - Unsaturated
 - Polyunsaturated
 - Neutral lipids
 - Phospholipids

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- **Proteins: the workhorses of the cell.**

Proteins are the most abundant and functionally versatile of the cellular macromolecules.

functions include structure, enzymes, mobility.

Proteins are formed from 20 different monomers, the **amino acids**. Linkages are through peptide bonds.

Amino acids-subunits of proteins

- 20 different amino acids
- all but one have the same basic structure (central carbon, to which is attached a carboxyl group and an amino group)
- the exception is proline (ring structure)

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[Lehninger, 1975]

Structure defines function.

Primary structure: the linear sequence of amino acids -peptide bond-condensation

Secondary structure: the peptide bond is relatively rigid, relatively little rotation so limited number of stable arrangements.

Tertiary structure- 3-dimensional arrangement; represents minimal energy state; usually sensitive to temperature, pH, salt concentration, disulfide bonds help to maintain conformation.

Quaternary structure- associations of multiple proteins. E.g., hemoglobin or antibodies are made of 4 separate protein subunits.

Proteins can combine with other types of molecules (glycoproteins, lipoproteins).

Enzymes are proteins that carry out chemical reactions involving small molecules by acting as **catalysts**. Tertiary structure brings reactants together, lowers free energy barrier, and accelerates reactions by many orders of magnitude.

Inorganic ions may be required as **cofactors** for enzymatic activity (e.g., zinc, copper, iron). “All (almost) enzymes are proteins, but not all proteins are enzymes.”

DNA: the cell’s master molecule.

Deoxyribonucleic acid or **DNA** is the macromolecule that garners the most public attention.

As we explore the interaction of radiation with biological material, DNA will assume central importance.

The structure of DNA was proposed in 1953 by James Watson and Francis Crick. DNA consists of two long helical strands that are wound together in a **double helix**.

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[Lodish, 2000]

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DNA structure

Nucleic acids (DNA and RNA) are linear polymers composed of monomers called **nucleotides**.

All nucleotides have a common structure: a **phosphate group** is linked by a **phosphodiester bond** to a **pentose sugar** (ribose in RNA and deoxyribose in DNA)

The **base** components of the nucleic acids are heterocyclic ring structures called **purines** or **pyrimidines**.

Purines: adenine, guanine

Pyrimidines: cytosine, thymine, uracil

The charge on the phosphate backbone gives nucleic acids a **net negative charge**.

Most nucleic acids in cells are **associated with proteins**.

Native DNA is a **double helix** of complementary antiparallel chains.

The two sugar-phosphate backbones are on the outside. The bases are on the inside connected by hydrogen bonds. **Base-pair complementarity** defines the base bonding:

A-T

C-G

Also known as **Watson-Crick base pairs**.

Each strand of DNA is composed of 4 different monomers called nucleotides.

[Image removed due to copyright considerations]

Note that adenosine and guanosine are both purines, and thymidine and cytidine are pyrimidines. A pairs with T, and G pairs with C.

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DNA is the **storage form of genetic information**.

DNA contains information about which proteins are to be made and when.

Proteins read the information in the DNA for use by the cell and translate it into a transportable unit called **messenger RNA (mRNA)**.

Other proteins read the mRNA sequence and assemble the amino acids into proteins.

This is the **central dogma** of biology: the coded genetic information hard-wired into DNA is transcribed into transportable cassettes, composed of mRNA, each mRNA cassette contains the information for synthesis of a particular protein.

DNA → mRNA → protein

Gene expression is the process of translating the information in the DNA into functional proteins.

Higher order structure: organizing DNA into chromosomes.

The total length of the DNA in a cell can be up to 100,000 times the cells diameter. Packaging the DNA is crucial to the cell architecture and function. The longest DNA molecules in human chromosomes ($2-3 \times 10^8$ base pairs) are almost 10 cm long!!

❖ *Eukaryotic nuclear DNA associates with histone proteins to form chromatin.*

Chromatin consists of an approximately equal mass of DNA and protein in a highly compact structure known as **chromatin**. The general structure of chromatin is similar in all eukaryotic cells.

The most abundant of the DNA-associated proteins are called **histones**. Histones are a family of basic (positively charged) proteins present in all eukaryotic cell nuclei. H1, H2A, H2B, H3 and H4

Histone sequences are highly conserved among species.

❖ *Chromatin exists in extended and condensed forms.*

Chromatin appearance depends on the conditions (primarily the salt concentration or ionic strength) used in the solutions to isolate it from the cell.

At low salt concentration chromatin appears like “beads on a string” or in an **extended form**.

The beadlike structures are called **nucleosomes**. Nucleosomes are composed of DNA and histones, are about **10 nm in diameter** and are the basic structural unit of chromatin.

At high salt concentration, isolated chromatin assumes a more **condensed fiberlike form** that is **30 nm in diameter**.

Structure of nucleosomes;

- A protein core with DNA wound on its surface like thread on a spool.
- Nucleosomes contain 146 base pairs wrapped slightly less than two turns around the protein core.
- The DNA of nucleosomes is more protected from enzymes (and radiation?) than the linker DNA between nucleosomes.

Structure of condensed chromatin:

- When extracted from cells in isotonic buffers, most chromatin appears as fibers, 30 nm in diameter.
- Nucleosomes are thought to be packed into a spiral or **solenoid arrangement**, with six nucleosomes per turn.

Modification of the histones (acetylation) can affect the binding and the assembly into condensed chromatin.

Condensed chromatin structure may be dynamic, with sections partially unfolding and then refolding.

Chromatin in regions not being transcribed exists in the condensed form, and possibly higher order structures built from it.

Chromatin in regions actively being transcribed is thought to exist in the extended “beads on a string” form.

Eukaryotic chromosomes contain one linear DNA molecule.

It is difficult to isolate high molecular weight DNA without breaking it.

[Image removed due to copyright considerations]

[Lodish, 2000]

Chromatin is further organized into chromosomes

Chromatin is further organized into large units hundreds of kilobases in length called **chromosomes**.

Chromosome number, size and shape at metaphase (the **karyotype**) are species specific.

In **non-dividing** cells the **chromosomes are not visible**.

During **mitosis** (and meiosis) the **chromosomes condense** and become visible.

Almost all cytogenetic work has been done on metaphase cells.

The condensation results from several orders of folding and coiling of the 30 nm chromatin fibers.

At mitosis, cells have progressed through S phase and duplicated the DNA.

Metaphase chromosomes are composed of two sister **chromatids**.

Non-histone proteins provide a scaffold for long chromatin loops.

Megabase-long loops of 30 nm DNA fibers are thought to associate with the flexible **chromosome scaffold**, yielding an extended form characteristic of chromosomes during interphase.

Coiling of the scaffold into a helix and further packing of this helical structure produces the highly condensed structure characteristic of **metaphase chromosomes**.

Genes are located primarily within **chromatin loops**. **Scaffold associated regions** serve as the attachment points of the loop to the protein scaffold.

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The Cell Cycle

- New cells arise when one cell divides or two cells (like sperm and egg) fuse.
- These events initiate a cell-replication program that is encoded in the DNA and executed by proteins.
- A period of cell growth, and replication of DNA is then followed by cell division.
- Cell growth and division is highly regulated in the body.
- Cancer occurs when a cell escapes from this regulation and growth is unchecked.

Most eukaryotic cells live according to an internal clock, they proceed through a series of phases called the **cell cycle**.

- **G₁**: the **gap** between the end of mitosis and beginning of S phase
- **S phase**: DNA is duplicated: cells have a DNA content that progressively increases from $2n$ to $4n$.
- **G₂**: time between the end of S and the beginning of mitosis
- **M**- mitosis: Cell division: the two daughter cells receive all of the genetic information of the parent cell.
- **G₀**: quiescent cells, not actively cycling.

G₁ and G₂ are periods of apparent inactivity between the major discernable events in the cell cycle.

- Bacteria can replicate their single chromosome and divide in about 20 minutes.
- Most mammalian cells have a cell cycle time on the order of 10-12 hours.
- Some cells (nerve cells, striated muscle cells) do not divide at all. They have temporarily exited from the cell cycle and entered a quiescent state called G₀.

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Checkpoints

“Proofreading before division”

Progress through the cell cycle is regulated at key **checkpoints** along the way that monitor the status of the cell.

Before entering mitosis, the integrity of the DNA is checked.

Exposure to radiation causes a block in G₂.

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Mitosis is the process for partitioning the genome equally during cell division. The **mitotic apparatus** captures the chromosomes and pulls them to the opposite sides of the dividing cell.

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- **Prophase:** the replicated chromosomes (each containing two identical chromatids) are condensed and released to the cytoplasm when the nuclear membrane breaks down.
- **Metaphase and anaphase:** the chromosomes are sorted and moved to opposite ends of the cell.
- **Telophase:** marks the end of mitosis as a membrane is reformed around each set of chromosomes.
- **Cytokinesis:** division of the cytoplasm and separation of the two daughter cells.

[Lodish, 2000]

What’s cancer?

Cancer-uncontrolled cell growth

- **Tumor**- mass formed by growing cancer cells
- **Malignant**- destructive, invasive, can metastasize
- **Benign**- slowly growing, does not invade surrounding tissues or metastasize, usually encapsulated.
- **Metastasis**- spread of the tumor cells to other sites, where they establish secondary areas of growth.
- **Angiogenesis**- both primary and secondary tumors require new blood vessels to sustain growth.

E.g.,

- **Carcinoma**- solid tumor arising from epithelial tissue such as skin, colon, lung, breast.
- **Sarcoma**- solid tumor arising from connective tissue such as bone.

Tumor	Tissue
<i>Malignant</i>	
Adenocarcinoma	Glandular
Carcinoma	Epithelial
Glioma	Glial cells in CNS
Hepatoma	Liver
Leukemia	WBCs leukocytes
Lymphoma	lymphocytes
Melanoma	Pigment cells
Myeloma	Plasma cells (bone marrow)
Nephroblastoma	Kidney
Neuroblastoma	Nerve cells
Retinoblastoma	Eye (retina)
Sarcoma	Connective tissue
Seminoma	Reproductive cells
Squamous	Epidermal
<i>Usually benign</i>	
Adenoma	Glandular
Chondroma	Cartilage
Fibroma	Fibroblasts
Osteoma	Bone
papilloma	Surface epithelia

Cancer cells can multiply in the absence of normally required growth factors and are resistant to signals that normally program cell death.

Malignant cells show many differences from normal counterparts.

- Less well differentiated
- More rapid growth
- Loss of normal attachments to neighbors, loss of contact inhibition
- Growth factor independent cell growth
- Genetically unstable (phenotype and genotype can change with time)
- Disruptions to cytoskeleton
- Higher metabolic rate
- Changes in cytoplasmic ion concentrations

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Genes involved in cancer

Seven types of proteins participate in the control of cell growth.

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Oncogenes- cancer causing genes

Many oncogenes are altered forms of normal cellular genes called proto-oncogenes
Gain-of-function mutations convert proto-oncogenes into oncogenes.

Tumor suppressor genes- genes which code for cell cycle control proteins

- Loss-of-function mutations in tumor suppressor genes are oncogenic.
- Usually act recessively, both copies must be deleted or mutated to lose the normal cell growth suppression effect.

Multi-step progression of cancer

Development of cancer requires several mutations

Consistent with the observation of increased incidence as a function of age

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- Cancer: a clone from a single cell?
- Colorectal carcinoma well studied with biopsy material and genetic analysis at all stages of development.
 - Visible on endoscopy
 - Biopsy material available
 - Analyze genetic mutations

Carcinogens- agents that can cause cancer

Viruses- can introduce oncogenes, or suppress genes that inhibit cell growth

- **Retroviruses-** RNA viruses, integrate into the genome of the infected cell as a DNA copy and can carry oncogenes derived from cellular proto-oncogenes.
- **DNA tumor viruses-** contain oncogenes of purely viral origin

Chemicals- usually thought to act as carcinogens by causing DNA damage.

- Some of the most potent are alkylating agents that are capable of adding organic groups to DNA.
- Others, (polycyclic hydrocarbons) become carcinogenic when they are biochemically modified in cells, often as the cell attempts detoxification
- Phorbol esters- do not cause DNA damage, but promote growth (may only work when DNA damage also occurs from another agent)

Radiation- also thought to act by causing DNA damage

- **UV-** is absorbed directly by DNA, causing base changes, in sunlight can lead to skin cancer.
- **Ionizing radiation** is actually a relatively weak carcinogen.

Apoptosis- now recognized that cancer is due not only to uncontrolled cell growth, but also to loss of control in regulated cell growth.

In an adult, normal tissues are in homeostasis- no net increase in cell numbers because of a balance between cell division and cell death due to apoptosis.

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Apoptosis vs. Necrosis

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