01.14.09
Lecture 2: How to Study Cells
Cellular Unity and Diversity

Cell theory: cells are the fundamental unit of life

- Delimiting membrane to separate inside from out
- Metabolism to build complex molecules from food and energetic molecules from light (photosynthesis) or respiration
- Capacity for reproduction:

Genes \(\Rightarrow\) transcription \(\Rightarrow\) translation \(\Rightarrow\) structure & regulation

DNA \(\Rightarrow\) mRNA \(\Rightarrow\) proteins \(\Rightarrow\) subunits & enzymes
Cellular Unity: all cells share a set of common characteristics

- Common biochemistry: all cells use the same basic building blocks (e.g. DNA, RNA, amino acids, lipids, carbohydrates)

- Common macromolecules and supramolecular structures and physiology (e.g. lipid bilayers, ribosomes, use ATP as energy)
Cellular Diversity: cells may be categorized based on their complexity

Two major classes of cells based on complexity of intracellular organization:

1. Prokaryotes
   (no nuclear envelope)
2. Eukaryotes
   (nuclear envelope)
A typical prokaryotic cell

**Capsule:** Layer of polysaccharide (sugars) that protects the bacterial cell. Often associated with pathogenic bacteria as it serves as a barrier against phagocytosis by white blood cells.

**Cell wall:** Composed of peptidoglycan (polysaccharides + protein). Maintains the overall shape and structure of the cell.

**Plasma membrane:** The external membrane. Regulates selective transport into and out of the cell.
A typical prokaryotic cell

**Flagella:** Stiff helical structure rotated by a rotary engine embedded in the membrane. Produces motility.

**Pili:** Hollow, hair-like structures that allow bacterial conjugation.

**Nucleoid:** DNA in the bacterial cell is generally confined to this region - not bounded by a membrane.

**Ribosomes:** The sites of protein synthesis - smaller than the ribosomes in eukaryotic cells.
A typical eukaryotic cell

**Plasma membrane:** The external membrane. Regulates selective transport into and out of the cell. Has proteins that serve as receptors and cell-cell connectors.

**Nucleus:** Houses most of the cell's genetic material as DNA packaged into chromatin. The site of DNA replication and gene transcription. Bound by a double membrane with access possible through nuclear pores.

**Mitochondria:** Composed of a double membrane (like the nucleus). Cellular "power factory" - major site of ATP synthesis. Possesses its own DNA and ribosomes (believed to be evolved from bacteria).
A typical eukaryotic cell

Ribosomes: Molecular machines that read mRNAs and direct protein synthesis. Soluble in cytosol and bound to Rough Endoplasmic Reticulum (RER)

Endoplasmic reticulum (ER): Site of lipid biosynthesis: Site of membrane protein synthesis (by RER-bound ribosomes) and of proteins destined for secretion

Golgi apparatus: Stacks of membrane-bound discs. Proteins coming from the ER are modified with sugars (glycosylation)

Lysosomes: Contain proteases and digestive enzymes. Fuse with endocytic vesicles to digest contents
A typical eukaryotic cell

**Peroxisomes:** Contain oxidative enzymes for lipid and amino acid metabolism. Hydrogen peroxide (very toxic) generated and degraded here.

**Cytosol:** A solution of large and small molecules - very dynamic.

**Cytoskeleton:** 3 types of filamentous polymers that act as a scaffolding to give cells shape and mechanical strength. Tracks for transport of organelles and machinery for cell division.

**Centrosome:** Organizes cytoskeleton. Pairs of centrioles + pericentriolar material (PCM).
## Prokaryotes vs. Eukaryotes

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<thead>
<tr>
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<th>Prokaryotes</th>
<th>Eukaryotes</th>
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<tbody>
<tr>
<td><strong>Organisms:</strong></td>
<td>Bacteria, cyanobacteria, archae</td>
<td>protists, fungi, plats, &amp; animals</td>
</tr>
<tr>
<td><strong>Cell size:</strong></td>
<td>~1-10 μm long</td>
<td>10-100 μm long</td>
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<tr>
<td><strong>Organelles:</strong></td>
<td>few or none</td>
<td>many</td>
</tr>
<tr>
<td><strong>DNA:</strong></td>
<td>circular, in cytoplasm</td>
<td>linear, in nucleus</td>
</tr>
<tr>
<td><strong>RNA &amp; protein:</strong></td>
<td>RNA and protein made in same compartment</td>
<td>RNA synthesized in nucleus</td>
</tr>
<tr>
<td></td>
<td>primitive cytoskeleton, no endocytosis, no exocytosis</td>
<td>protein synthesized in cytoplasm</td>
</tr>
<tr>
<td><strong>Cytoplasm:</strong></td>
<td></td>
<td>complex cytoskeleton, endocytosis, exocytosis</td>
</tr>
<tr>
<td><strong>Cell division:</strong></td>
<td>by binary fission</td>
<td>by mitosis or meiosis</td>
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<tr>
<td><strong>Organization:</strong></td>
<td>mainly unicellular</td>
<td>unicellular or multicellular</td>
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<tr>
<td></td>
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<td>with differentiation of many cell types</td>
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What kind of cells to study?
Experimental organisms are valuable for the study of cell and developmental biology

<table>
<thead>
<tr>
<th>Organism</th>
<th>Description</th>
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<tr>
<td><strong>Yeast</strong> (<em>Saccharomyces cerevisiae</em>)</td>
<td>Control of cell cycle and cell division, Protein secretion and membrane biogenesis, Function of the cytoskeleton, Cell differentiation, Aging, Gene regulation and chromosome structure</td>
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<tr>
<td><strong>Mice</strong>, including cultured cells</td>
<td>Development of body tissues, Function of mammalian immune system, Formation and function of brain and nervous system, Models of cancers and other human diseases, Gene regulation and inheritance, Infectious disease</td>
</tr>
<tr>
<td><strong>Plant</strong> (<em>Arabidopsis thaliana</em>)</td>
<td>Development and patterning of tissues, Genetics of cell biology, Agricultural applications, Physiology, Gene regulation, Immunity, Infectious disease</td>
</tr>
<tr>
<td><strong>Roundworm</strong> (<em>Caenorhabditis elegans</em>)</td>
<td>Development of the body plan, Cell lineage, Formation and function of the nervous system, Control of programmed cell death, Cell proliferation and cancer genes, Aging, Behavior, Gene regulation and chromosome structure</td>
</tr>
<tr>
<td><strong>Fruit fly</strong> (<em>Drosophila melanogaster</em>)</td>
<td>Development of the body plan, Generation of differentiated cell lineages, Formation of the nervous system, heart, and musculature, Programmed cell death, Genetic control of behavior, Cancer genes and control of cell proliferation, Control of cell polarization, Effects of drugs, alcohol, pesticides</td>
</tr>
<tr>
<td><strong>Zebrafish</strong></td>
<td>Development of vertebrate body tissues, Formation and function of brain and nervous system, Birth defects, Cancer</td>
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Cultured animal cells are an experimentally tractable system

- Primary culture: cells are taken directly from the organism and grown. These cultures only divide for a certain number of time, then quit.

- Cell lines: derived from undifferentiated embryonic cells or tumor cells. These cultures are immortal.

- Cultured cells are grown in medium.
Pros and cons of cultured cells as a model

Advantages:
1. Most tissues consist of multiple cell types, whereas cells of a specific type with homogeneous properties can be grown in culture.
2. Experimental conditions are easier to manipulate and observe with cultured cells than with organisms.

A BIG disadvantage:
1. Cells cultured in a dish are not in their normal environment and their behaviors are not regulated by other cells and tissues the way they are in an organism.
Cells are (relatively) small. How do we study them?
Light microscopy led to the discovery of cells

The microscope used by Robert Hook, the first person to discover cells in thin sections of cork
A modern light microscope
What microscopes do - designed for:

1. **Magnification**: the relative enlargement of the specimen when viewed through the microscope

   ![Empty Magnification](image)

   2 Pictures of a flea at same magnification. (a) was acquired using optics that provided higher resolution than (b)

2. **Resolution**: the ability to discern fine details. The resolution of a microscope is determined by the wavelength of light (or energy) used for illumination. For light microscopy, the limit is \( \sim 200 \text{ nm} \).

3. **Contrast**: the difference in intensity between the image and the background. Contrast is produced in the specimen by staining with colored dyes that absorb light, by using special optical techniques, or
Cells and tissues must be processed for light microscopy

1. Specimens must be preserved and “glued” together in a process called fixation.

2. Thick specimens (such as intact tissues) are embedded in a solid matrix (paraffin or plastic) and cut into thin sections.
3. Sections may then be stained with dyes to highlight certain cells or parts of cells and viewed by light microscopy.
Living cells can be visualized using techniques that generate contrast by refraction of light

- Most cells and tissues are transparent and colorless (low contrast)
- Cellular components differ from each other in refractive index
- Optical techniques such as phase-contrast and differential interference contrast can be used to view living, unstained cells
Fluorescence microscopy exploits special molecules called fluorophores

1. Fluorescent dyes/fluorophores absorb light (energy) of a specific color (wavelength)

2. Absorbtion excites them into a high energy state.

3. This energy is reemitted as light of a longer wavelength (less energy).

4. The chemical properties of a dye determine its excitation and emission wavelengths. Many different dyes available.

Fluorescein
Examples of multi-color fluorescence imaging

Living endothelial cell stained to show ER (green) and mitochondria (red)

Fixed epithelial cell stained to show actin (purple), DNA (blue), and the Golgi (green)
Antibodies

- Antibodies are composed of 2 X heavy chains, 2 X light chains
- Bind with very high affinity to their target antigen
- May be produced in mice, rabbits, goats, sheep, chickens
The light path of a fluorescence microscope
Confocal microscopy is a special type of fluorescence microscopy used for thick specimens.

(a) Conventional fluorescence microscopy

(b) Confocal fluorescence microscopy
Green fluorescent protein (GFP)

- A natural protein fluorophore expressed in jellyfish
- Absorbs blue light and emits (fluoresces) green light
- DNA sequence can be fused to other genes and reintroduced in to cells
- Its discoverers won the 2008 Nobel Prize in Chemistry
GFP can be used in living animals
Electron microscopy allows high resolution imaging of biological structures

The principles of electron microscopy are similar to light microscopy except:

• Beams of electron are used instead of light
• Electron beam has a wavelength of 0.01 nm providing a resolution of 1-2 nm (TEM) or 10-20 nm (SEM)
• The lenses used to focus the electron beams are electromagnets rather than glass lenses
Transmission electron microscopy

- Samples must be fixed and embedded in plastic resin
- Embedded samples are cut into thin sections ~70 nm thick on a diamond blade
- To provide contrast, specimen sections must be treated with heavy metals (osmium and uranium)
- Electrons pass through the sample except for regions that are stained with metals
Transmission electron microscopy
Scanning electron microscopy

• Specimens are fixed and coated on their surface with an electron-dense layer of metal
• Electrons are reflected off the surface on to a detector
Scanning electron microscopy

Red blood cells

Tracheal cilia
Antibodies to cellular components may be used for localization by fluorescence & EM

Immunofluorescence staining for Golgi (blue), actin (green) histones (red)

Immunogold for catalase