Cellular Organization

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• Organelles

• Distribution of organelles by microtubules and motor proteins

• Signal sequences

• Importing proteins into organelles
Cells contain a variety of organelles that perform specific and unique functions.

Organelles are collections of specific proteins and other components. Each type of organelle performs a unique set of biochemical reactions. Organelles increase the efficiency of these reactions by concentrating the proteins and substrates of a common pathway in a small environment. Some organelles are surrounded by a membrane similar to the cell membrane which helps create a specific internal environment. For example, the lysosome has an acidic pH compared to the cytoplasm.
Lysosomes contain a variety of digestive enzymes that would chew up cell components.

Another advantage of organelles is that they protect cells from noxious molecules. The lysosome contains many digestive enzymes that breakdown old or foreign macromolecules, including protein, nucleic acids and carbohydrates. If these enzymes were released to the cytoplasm, they could digest critical cellular components. By housing these enzymes in a membrane-bound organelle, the cell not only increases the efficiency of the digestive reactions but also protects the rest of the cell.
Organization of the cytoplasm: microtubules and actin filaments
Cells use microtubules to position organelles within their cytoplasm.

Cells organize and localize organelles within their cytoplasm by moving them to intracellular locations where they are needed. For example, cells will position mitochondria at sites of greatest energy consumption. To position organelles, cells need a mechanism to transport organelles through the cytoplasm and then anchor them at specific sites. Microtubules play a critical role in organizing cytoplasm of cells as shown in these images. Microtubules are in red and Golgi, a membrane-bound organelle, is in green. Cells utilize microtubules to tether Golgi in specific location in the cell, usually adjacent to the nucleus. If we disrupt the microtubules with a drug, the Golgi fragment and lose their positioning.
Microtubules extend throughout the cytoplasm.

Microtubules are effective for organizing cells because they are long filaments that extend throughout cytoplasm. They are large enough to span most cells and allow cells to position organelles in specific regions. Microtubules are labeled in green.
Microtubules are a hollow tube of tubulin dimers. Both bind the nucleotide GTP. Heterodimers assemble into filaments end on end to form protofilaments. 13 protofilaments interact to make a single microtubule. The arrangement of the protofilament creates a long, hollow tube similar to a PVC pipe. The extensive lateral contacts between subunits in neighboring protofilaments gives microtubules greater strength and allows them to grow longer than actin filaments. Microtubules are also polarized with one end, the minus end, containing an exposed alpha subunit and the other end, the plus end, having an exposed beta subunit. Microtubules grow in length from their plus ends, but their minus ends are unstable and are usually associated with proteins that prevents subunits from depolymerizing from the filament.
Microtubules grow from their plus ends.

Video shows growth of microtubules in vitro. Microtubules grow from their plus ends as more dimers are added to the filament. Occasionally, microtubule will stop growing and then shrink as dimers fall off of plus end. Inside cells, the plus ends of microtubules are capped or stabilized so they don't shrink. The repeated growing and shrinking allow microtubules to explore different areas of the cell and cells can reposition or reorient microtubules. Note that the minus ends of the microtubules emanate from a common center. This region is called the microtubule organizing center and stabilizes the minus ends of microtubules.
Proteins link organelles to microtubule network.

The mitotic organizing center (MTOC) stabilizes the minus ends of microtubules in most cells. The MTOC contains a large number of proteins including a pair of organelles called centrioles. Centrioles are an example of an organelle that is not membrane-bound. The location of the MTOC determines the orientation of microtubules in a cell. If the MTOC is located in the center of the cell, the microtubules radiate outward toward the cell membrane with their plus ends at the cell membrane.
Kinesins and dynein mediate bidirectional transport of organelles along microtubules.

Microtubules also mediate the transport of organelles, proteins and nucleic acids within the cytoplasm of cells. Microtubules-based motor proteins, kinesins and dynein, use microtubules to move cellular material within cells. Kinesins comprise a large family of motor proteins most of which move toward the plus ends of microtubules. In contrast, dynein moves toward the minus ends. All motor proteins contain a domain that binds microtubules and hydrolyzes ATP. The energy released through ATP hydrolysis propels the motor protein along a microtubule. Motor proteins that transport cellular material also contain a domain that interacts specifically with an organelle, protein or nucleic acid. By controlling the type of motor protein is attached to an organelle, cells control the location of that organelle.
Cells regulate activity of motor proteins to control distribution of organelles.

Organelles will often have both types of motors on their surface, allowing cells to adjust their position. For example, these melanocytes contain a pigmented organelle called a melanosome. In some organisms, melanocytes position melanosomes in response to the amount of light. In the presence of light, kinesin on the surface of melanosomes moves them to the periphery of cells. In the dark, dynein returns the melanosomes to the center of the cell.
Actin filaments concentrate at the plasma membrane in many cells.

Actin filaments show a different distribution than microtubules. Actin filaments usually localize near the cell membrane or at sites where cells attach to another cell or external surface. Actin filaments provide structural support to the cell membrane and allow cells to generate tension on the cell membrane which can lead to cell contraction.
Actin filaments are a polymer of a single globular protein of about 43 kD. Actin monomers bind and hydrolyze ATP and have an asymmetric structure as one side of the protein contains the pocket to bind ATP. Actin monomers polymerize into filaments in a helical fashion. Actin filaments appear to be of two single filaments wrapped around each other. The lateral interactions between monomers allows filaments to grow to greater lengths than if the filament were a single, straight chain of monomers. Individual actin filaments are shorter and less rigid than microtubules.
Some types of myosins transport vesicles and organelles.

Similar to microtubules, actin filaments also support the transport of organelles, proteins and nucleic acids. Instead of kinesins and dynein, actin filaments use myosins to transport intracellular organelles and other cellular material. These myosins resemble the structure of kinesin in that contain motor domains that bind actin filaments and hydrolyze ATP and a domain at their C-terminus that links the motor to different cargo. The motor domains of myosin generate force along actin filaments to move themselves within the cytoplasm.
Microtubules support long distance transport; actin filaments transport near the cell membrane.

Because actin filaments are shorter than microtubules, myosins usually transport organelles over shorter distances compared to kinesins and dynein. Myosin-based transport is often used near the plasma membrane, an area rich in actin filaments. One model of how kinesins and myosins work together is that kinesins transport organelles from the center of the cell towards the periphery, where myosins take over moving organelles near the plasma membrane. For example, a secretory vesicle might be transported from the trans-Golgi network towards the plasma membrane along microtubules. When the secretory vesicle reached the actin filaments underneath the plasma membrane, myosins would complete the transport of the vesicle to the plasma membrane.
Targeting proteins to specific organelles
Proteins synthesized in cytoplasm must get to correct organelle.

Each organelle contains a unique set of proteins and other components that allows that organelle to perform its biochemical functions. Cells need a mechanism to target certain proteins to one type of organelle. In addition, because many organelles are surrounded by a membrane, there must be a way for proteins to cross the membrane of an organelle.
Proteins are imported into organelles from cytosol or transported between organelles.

Signal sequences target proteins to specific organelles. Signal sequences are usually one or more stretches of amino acids within a protein that is recognized by machinery that delivers that protein to an organelle. The synthesis of all proteins starts in the cytosol, but those proteins with signal sequences are delivered to their appropriate organelle. Many organelles receive proteins through the secretory pathway. Proteins are initially translated and inserted into the ER. Proteins are transported to the Golgi and then delivered to the different organelles. Some organelles receive proteins directly from the cytosol. The nucleus is a unique organelle as it allows bidirectional movement of proteins.
Proteins contain signal sequences that determine their final destination.

<table>
<thead>
<tr>
<th>Function of Signal Sequence</th>
<th>Example of Signal Sequence</th>
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<tbody>
<tr>
<td>Import into nucleus</td>
<td>-Pro-Pro-Lys-Lys-Lys-Lys-Lys-Lys-Val-</td>
</tr>
<tr>
<td>Export from nucleus</td>
<td>-Leu-Ala-Leu-Lys-Leu-Ala-Gly-Leu-Asp-Ile</td>
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<tr>
<td>Import into mitochondria</td>
<td>$^+\text{H}_3\text{N-Met-Leu-Ser-Leu-Arg-Gln-Ser-Ile-Arg-Phe-Phe-Lys-Pro-Glu-Arg-Asn-Thr-Leu-Cys-Ser-Ser-Arg-Tyr-Leu-Leu}$</td>
</tr>
<tr>
<td>Import into ER</td>
<td>$^+\text{H}_3\text{N-Met-Met-Ser-Phe-Val-Ser-Leu-Leu-Leu-Val-Gly-Ile-Leu-Phe-Trp-Ala-Thr-Glu-Ala-Glu-Gln-Leu-Thr-Lys-Cys-Glu-Val-Phe-Gln}$</td>
</tr>
<tr>
<td>Import into peroxisomes</td>
<td>-Ser-Lys-Leu-COO-</td>
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Signal sequences that target proteins to the different organelles have been identified. Some signal sequences use a nearly identical stretch of amino acids in different proteins to target those proteins to a specific organelles. Other signal sequences use the properties of amino acids (hydrophobic, acidic, basic) to target proteins to an organelle. The amino acids in these signal sequences will often vary between proteins.
Small changes in signal sequences have profound effects on location of proteins.

Slide illustrates how subtle changes in signal sequences can affect the localization of proteins. In humans, a certain nucleoside transporter contains a 4 amino acid motif that targets it to mitochondria. In mice, the same protein contains two changes in this motif and this targets the protein to the plasma membrane. If you experimentally change the motif in the human protein to the mouse version, the human transporter localizes to the plasma membrane.
Fialuridine is a uridine analog that is a potent inhibitor of hepatitis B.

In the early 1990’s there was a clinical trial of a drug designed to treat hepatitis B in 15 patients. Hepatitis B is a virus that affects the liver, causing inflammation. One common treatment for this type of virus is nucleoside analogs. Nucleoside analogs are similar to the nucleosides that are used to synthesize DNA, but the analogs are altered so that they stop synthesis or affect the stability of the DNA. Fialuridine is a nucleoside analog that was found to be effective in reducing hepatitis B in animal models. It was subsequently used in human trials and initially it appeared to reduce the amount of virus. But then something went terribly wrong. Long after the clinical trials were concluded, researchers discovered something unique about the way fialuridine affects human cells. Because humans have a certain nucleoside transporter that localizes to mitochondria, fialuridine can enter mitochondria. Why is this bad? Mitochondria contain their own DNA genome and require expression of genes in their genome to function properly. By preventing replication of the mitochondria genome, fialuridine poisoned mitochondria, reducing their ability to generate ATP. Why wasn’t this discovered before the clinical trials? Fialuridine was tested on animal models for safety, but those animals were mice and rats whose nucleoside transporter lacks the mitochondrial signal sequence. Consequently, their mitochondria did not take up fialuridine and were not affected by the drug.

What happened in the clinical trials? By reducing the activity of mitochondria, fialuridine forced energy-intensive cells to start using glycolysis to generate ATP. This lead to an increase in lactate in the blood and a condition called lactic acidosis. Because the liver processes lactate, the increase production of lactate led to liver failure 7 patients, 5 of whom died.
Importing proteins into membrane-bound organelles

So we've seen how proteins get targeted to a membrane-bound organelle but how are they imported into that organelle. Proteins can’t diffuse across the membrane so they need a way to get across. Organelles that directly import proteins from the cytoplasm have proteins that form pores in their membrane. These pores allow proteins to transit from the cytoplasm into the organelle. We’re going to look at two different types of pores.
Most proteins imported co-translationally into the ER through the translocon.

The ER contains a set of proteins called the translocon that form a pore in the membrane through which proteins will be threaded from the cytoplasm into the ER. Two important notes about the process of moving a protein from the cytoplasm to ER. First, the translocation of proteins is usually coupled to their synthesis. As mRNAs are being translated the newly made protein is being inserted into the translocon. The energy used during translation can push the protein through the translocon. The second important point is that proteins cross the ER in an unfolded state. The translocon is not large enough to accommodate folded proteins. Consequently, proteins must fold into their 3 dimensional structure in the ER. As we will see in future lectures, the need for proteins to fold in the lumen has some important medical consequences. In this cartoon the signal sequence of the inserted protein is cleaved, generating a protein that resides in the lumen. But we also need a way to make integral membrane proteins.
Integral membrane proteins contain stop transfer sequence.

Integral membrane proteins contain a stretch of hydrophobic residues downstream from the signal sequence that function as stop transfer sequence. The stop transfer sequence halts the translocation of the protein. Eventually the protein will exit from the translocon and the stop transfer sequence will function as the transmembrane domain of the protein.
Nuclear pores are size selective channels.

The nucleus also uses a pore to import proteins, but uses a different mechanism than the ER. First, nuclear pores are large and can accommodate folded proteins. In fact, they can accommodate large multi-protein complexes such as ribosomes. The pores are so large than small proteins (< 30 kDa) can freely diffuse through the pore.
How do proteins larger than 30 kD get into the nucleus. These proteins have a signal sequence called the nuclear localization sequence. The NLS is recognized in the cytoplasm by a protein called importin. Importin binds the NLS and then guides the proteins to the nuclear pore. The complex then migrates through the pore to enter the nucleus. Inside the nucleus the complex is dissociated allowing the imported proteins to perform its function in the nucleus.
Protein import into peroxisomes and Zellweger’s Syndrome
Peroxisomes detoxify chemicals, metabolize fatty acids and synthesize key lipid.

Peroxisomes perform several vital functions. They metabolize many harmful molecules, such as phenols, formaldehyde and ethanol. They metabolize fatty acids into acetyl CoA which can be used in metabolic pathways to generate ATP. Finally, peroxisomes catalyze the initial step in the synthesis of a special lipid called plasmalogen. Plasmalogen localizes to the cell membrane of cells that form myelin sheaths around axons.
Proteins inserted post-translationally into peroxisomes.

Protein import into peroxisomes is similar to other membrane-bound organelles in that peroxisomal proteins contain a signal sequence that is recognized by proteins that deliver the peroxisomal protein to the peroxisome. Another set of proteins in the peroxisome membrane forms a channel through which peroxisomal proteins enter a peroxisome. The proteins that target proteins to peroxisomes and form the channel are called Pex proteins.
Mutations in Pex proteins lead to loss of peroxisomes.

Cells that contain mutations in the Pex genes can’t import proteins into peroxisomes. Consequently, the protein content of peroxisomes can’t be maintained and the cell loses its peroxisomes.
Pex mutations cause Zelleweger Syndrome and demyelination of nerves.

There are several diseases, termed Zelleweger Syndrome, that are caused by mutations in the genes that encode the Pex proteins. These mutations lead to a loss of peroxisomes. The consequence is a build up of toxic material in cells, especially in the liver, and an inability to fully myelinate the axons of neurons. The lack of myelinated axons affects motor neurons and infants with Zelleweger Syndrome have low muscle tone, show an inability to move and often fail to suckle. The prognosis of infants with Zelleweger Syndrome is poor and most die by 6 months.
Take home points...

- Organelles increase efficiency of biochemical reactions and protect cells from noxious molecules.
- Cells control the distribution of organelles through motor proteins and the cytoskeleton.
- Signal sequences guide proteins to specific organelles where protein machinery imports them into the organelle.