Lecture 3

I. Membrane Proteins
II. Intracellular Compartments
III. Protein Translocation

Ref: *MBoC* (5th Edition), Alberts • Johnson • Lewis • Raff • Roberts • Walter

Chapter 10 Membrane Structure
Chapter 12 Intracellular Compartments and Protein Sorting

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An experiment demonstrating the diffusion of proteins in the plasma membrane of mouse–human hybrid cells.

The mouse and human proteins are initially confined to their own halves of the newly formed heterocaryon plasma membrane, but they intermix over time. The two antibodies used to visualize the proteins can be distinguished in a fluorescence microscope because fluorescein is green and rhodamine is red.

Figure 10-35
A specific protein of interest can be expressed as a fusion protein with green fluorescent protein (GFP). In the FRAP technique, fluorescent molecules are bleached in a small area using a laser beam. The fluorescence intensity recovers as the bleached molecules diffuse away and unbleached molecules diffuse into the irradiated area. The diffusion coefficient is calculated from a graph of the rate of recovery: the greater the diffusion coefficient (mobility) of the membrane protein, the faster the recovery.
In the FLIP technique, a small area of membrane is **irradiated continuously**, and **fluorescence is measured in a separate area**. Fluorescence in the second area progressively decreases as fluorescent proteins diffuse out and bleached molecules diffuse in; eventually, all of the fluorescent protein molecules are bleached, as long as they are mobile and not permanently anchored to the cytoskeleton or extracellular matrix.
Cells Can Confine Proteins and Lipids to Specific Domains Within a Membrane

Four ways of restricting the lateral mobility of specific plasma membrane proteins:
(A) The proteins can self-assemble into large aggregates (e.g. bacteriorhodopsin in the purple membrane of Halobacterium);
(B, C) they can be tethered by interactions with assemblies of macromolecules (B) outside or (C) inside the cell;
(D) they can interact with proteins on the surface of another cell.

In an epithelial cell, protein A and protein B can diffuse laterally in their own domains but are prevented from entering the other domain, at least partly by the specialized cell junction called a tight junction.

Lipid molecules in the outer (noncytosolic) monolayer of the plasma membrane are likewise unable to diffuse between the two domains; lipids in the inner (cytosolic) monolayer, however, are able to do so (not shown). The basal lamina is a thin mat of extracellular matrix that separates epithelial sheets from other tissues.
The Spectrin-based cytoskeleton on the cytosolic side of the human red blood cell plasma membrane

(A) Spectrin dimers are linked together into a netlike meshwork by “junctional complexes”. The junctional complexes are composed of short actin filaments, band 4.1, adducin, and a tropomyosin molecule. The cytoskeleton is linked to the membrane through two transmembrane proteins (band 3 & glycophorin).

(B) The EM shows the cytoskeleton on the cytosolic side of a red blood cell membrane after fixation and negative staining.
The cytoplasm consists of the cytosol and the cytoplasmic organelles.
Table 12–1 Relative Volumes Occupied by the Major Intracellular Compartments in a Liver Cell (Hepatocyte)

<table>
<thead>
<tr>
<th>INTRACELLULAR COMPARTMENT</th>
<th>PERCENTAGE OF TOTAL CELL VOLUME</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cytosol</td>
<td>54</td>
</tr>
<tr>
<td>Mitochondria</td>
<td>22</td>
</tr>
<tr>
<td>Rough ER cisternae</td>
<td>9</td>
</tr>
<tr>
<td>Smooth ER cisternae plus Golgi cisternae</td>
<td>6</td>
</tr>
<tr>
<td>Nucleus</td>
<td>6</td>
</tr>
<tr>
<td>Peroxisomes</td>
<td>1</td>
</tr>
<tr>
<td>Lysosomes</td>
<td>1</td>
</tr>
<tr>
<td>Endosomes</td>
<td>1</td>
</tr>
</tbody>
</table>
### Table 12–2 Relative Amounts of Membrane Types in Two Kinds of Eucaryotic Cells

<table>
<thead>
<tr>
<th>MEMBRANE TYPE</th>
<th>PERCENTAGE OF TOTAL CELL MEMBRANE</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>LIVER HEPATOCYTE*</td>
<td>PANCREATIC EXOCRINE CELL*</td>
</tr>
<tr>
<td>Plasma membrane</td>
<td>2</td>
<td>5</td>
</tr>
<tr>
<td>Rough ER membrane</td>
<td>35</td>
<td>60</td>
</tr>
<tr>
<td>Smooth ER membrane</td>
<td>16</td>
<td>&lt;1</td>
</tr>
<tr>
<td>Golgi apparatus membrane</td>
<td>7</td>
<td>10</td>
</tr>
<tr>
<td>Mitochondria</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Outer membrane</td>
<td>7</td>
<td>4</td>
</tr>
<tr>
<td>Inner membrane</td>
<td>32</td>
<td>17</td>
</tr>
<tr>
<td>Nucleus</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Inner membrane</td>
<td>0.2</td>
<td>0.7</td>
</tr>
<tr>
<td>Secretory vesicle membrane</td>
<td>not determined</td>
<td>3</td>
</tr>
<tr>
<td>Lysosome membrane</td>
<td>0.4</td>
<td>not determined</td>
</tr>
<tr>
<td>Peroxisome membrane</td>
<td>0.4</td>
<td>not determined</td>
</tr>
<tr>
<td>Endosome membrane</td>
<td>0.4</td>
<td>not determined</td>
</tr>
</tbody>
</table>

*These two cells are of very different sizes: the average hepatocyte has a volume of about 5000 μ.m³ compared with 1000 μ.m³ for the pancreatic exocrine cell. Total cell membrane areas are estimated at about 110,000 μ.m² and 13,000 μ.m², respectively.*
Evolutionary Origins Explain the Topological Relationships of Organelles

Hypothetical schemes for the evolutionary origins of some organelles. The origins of mitochondria, chloroplasts, ER, and the cell nucleus might explain the topological relationships of these compartments in eucaryotic cells.

(A) A possible pathway for the evolution of the cell nucleus and the ER.

(B) Mitochondria (and plastids) are thought to have originated when a bacterium was engulfed by a larger pre-eucaryotic cell. This could explain why they contain their own genomes and why the lumens of these organelles remain isolated from the membrane traffic that interconnects the lumens of many other intracellular compartments.
Topologically equivalent spaces are shown in red. In principle, cycles of membrane budding and fusion permit the lumen of any of these organelles to communicate with any other and with the cell exterior by means of transport vesicles. Blue arrows indicate the extensive outbound and inbound vesicular traffic.

*Some organelles, most notably mitochondria and (in plant cells) plastids, do not take part in this communication and are isolated from the traffic between organelles shown here.
Proteins can move from one compartment to another by gated transport (red), transmembrane transport (blue), or vesicular transport (green). The sorting signals that direct a given protein’s movement through the system, and thereby determine its eventual location in the cell, are contained in each protein’s amino acid sequence. The journey begins with the synthesis of a protein on a ribosome in the cytosol and terminates when the protein reaches its final destination. At each intermediate station (boxes), a decision is made as to whether the protein is to be retained in that compartment or transported further. In principle, a sorting signal could be required for either retention in or exit from a compartment.

A Simplified “Roadmap” of Protein Traffic

Figure 12-6
Signal sequence mediated intracellular protein translocation

Table 12–3 Some Typical Signal Sequences

<table>
<thead>
<tr>
<th>FUNCTION OF SIGNAL SEQUENCE</th>
<th>EXAMPLE OF SIGNAL SEQUENCE</th>
</tr>
</thead>
<tbody>
<tr>
<td>Import into nucleus</td>
<td>-Pro-Pro-Lys-Lys-Lys-Arg-Lys-Val-</td>
</tr>
<tr>
<td>Export from nucleus</td>
<td>-Leu-Ala-Leu-Lys-Leu-Ala-Gly-Leu-Asp-Ile-</td>
</tr>
<tr>
<td>Import into mitochondria</td>
<td>^H,N-Met-Leu-Ser-Leu-Arg-Gln-Ser-Ile-Arg-Phe-Phe-Lys-Pro-Ala-Thr-Arg-Thr-Leu-Cys-Ser-Ser-Arg-Tyr-Leu-Leu-</td>
</tr>
<tr>
<td>Import into plastid</td>
<td>^H,N-Met-Val-Ala-Met-Ala-Met-Ala-Ser-Leu-Gln-Ser-Ser-Met-Ser-Ser-Leu-Ser-Leu-Ser-Ser-Asn-Ser-Phe-Leu-Gly-Gln-Pro-Leu-Ser-Pro-Ile-Thr-Leu-Ser-Pro-Phe-Leu-Gln-Gly-</td>
</tr>
<tr>
<td>Import into peroxisomes</td>
<td>^Ser-Lys-Leu-COO^-</td>
</tr>
<tr>
<td>Import into ER</td>
<td>^H,N-Met-Met-Ser-Phe-Val-Ser-Leu-Leu-Leu-Leu-Gly-Ile-Leu-Phe-Trp-Ala-Thr-Glu-Ala-Glu-Gln-Leu-Thr-Lys-Glu-Val-Phe-Gln-</td>
</tr>
<tr>
<td>Return to ER</td>
<td>^Lys-Asp-Glu-Leu-COO^-</td>
</tr>
</tbody>
</table>

Some characteristic features of the different classes of signal sequences are highlighted in color. Where they are known to be important for the function of the signal sequence, positively charged amino acids are shown in red and negatively charged amino acids are shown in green. Similarly, important hydrophobic amino acids are shown in white and hydroxylated amino acids are shown in blue. ^H,N indicates the N-terminus of a protein; COO^- indicates the C-terminus.

From Günter Blobel
Signal Sequences Direct Proteins to the Correct Cell Addresses

Most protein sorting signals reside in a stretch of amino acid sequence, typically 15–60 residues long. These **signal sequences** are often found at the N-terminus; specialized **signal peptidases** remove the signal sequence from the finished protein once the sorting process is complete. Signal sequences can also be internal stretches of amino acids, which remain part of the protein. In some cases, sorting signals are composed of multiple internal amino acid sequences that form a specific three-dimensional arrangement of atoms on the protein’s surface, called a **signal patch**.

* Each signal sequence specifies a particular destination in the cell.

![Diagram of signal sequences and signal patches](image)

Figure 12-8. Molecular Biology of the Cell, 4th Edition.
The Endoplasmic Reticulum (ER)

All eucaryotic cells have an ER. Its membrane typically constitutes more than half of the total membrane of an average animal cell. The ER is organized into a netlike labyrinth of branching tubules and flattened sacs that extends throughout the cytosol.

(A) Part of the ER network in a cultured mammalian cell, **stained with an antibody** that binds to a protein retained in the ER.

(B) Part of an ER network in a living plant cell that was genetically engineered to **express a fluorescent protein** in the ER.
Mammalian cells import most proteins into the ER co-translationally, whereas import proteins into mitochondria, chloroplasts, nuclei, and peroxisomes post-translationally.
The ER Is Structurally and Functionally Diverse

(A) An EM of the rough ER in a pancreatic exocrine cell that makes and secretes large amounts of digestive enzymes every day.

(B) Abundant smooth ER in a steroid-hormone-secreting cell.

(C) A 3-D reconstruction of a region of smooth ER and rough ER in a liver cell.
The Isolation of Purified Rough and Smooth Microsomes from the ER

(A) A thin section electron micrograph of the purified rough ER fraction shows an abundance of ribosome-studded vesicles.

(B) When sedimented to equilibrium through a gradient of sucrose, the two types of microsomes separate from each other on the basis of their different densities.

Figure 12-37
Signal Sequences Were First Discovered in Proteins Imported into the Rough ER

The signal hypothesis. A simplified view of protein translocation across the ER membrane, as originally proposed.

When the ER signal sequence emerges from the ribosome, it directs the ribosome to a translocator on the ER membrane (a pore in the membrane through which the polypeptide is translocated). A signal peptidase is closely associated with the translocator and clips off the signal sequence during translocation, and the mature protein is released into the lumen of the ER immediately after synthesis. The translocator is closed until the ribosome has bound, so that the permeability barrier of the ER membrane is maintained at all times.

For the history of the Signal Hypothesis, please listen to the Blobel’s Nobel lecture: http://nobelprize.org/nobel_prizes/medicine/laureates/1999/blobel-lecture.html
A Signal-Recognition Particle (SRP) Directs ER Signal Sequences to a Specific Receptor in the Rough ER Membrane

The SRP binds to both the exposed ER signal sequence and the ribosome, thereby inducing a pause in translation. The SRP receptor in the ER membrane, which is composed of two different polypeptide chains, binds the SRP–ribosome complex and directs it to the translocator.

Because one of the SRP proteins and both chains of the SRP receptor contain GTP-binding domains, it is thought that conformational changes that occur during cycles of GTP binding and hydrolysis ensure that SRP release occurs only after the ribosome has become properly engaged with the translocator in the ER membrane.
The Signal-Recognition Particle (SRP)

(A) A mammalian SRP is a rodlike complex containing **six protein subunits and one RNA molecule**. The SRP RNA forms the backbone that links the domain of the SRP containing the signal sequence binding pocket to the domain responsible for pausing translation. The 3-D outline of the SRP (shown in gray) was determined by cryo-electron microscopy. Where known, crystal structures of individual SRP pieces are fitted into the envelope and shown as ribbon diagrams. A bound signal sequence is shown as a green helix.

(B) SRP bound to the ribosome visualized by cryo-electron microscopy. SRP binds to the large ribosomal subunit so that its signal sequence binding pocket is positioned near the nascent chain exit site and its translational pause domain is positioned at the interface between the ribosomal subunits, where it interferes with elongation factor binding.
Free and Membrane-Bound Ribosomes

**FREE RIBOSOME CYCLE**

mRNA encoding a cytosolic protein remains free in cytosol

**MEMBRANE-BOUND RIBOSOME CYCLE**

ER signal sequence

mRNA encoding a protein targeted to ER remains membrane-bound

polyribosome bound to ER membrane by multiple nascent polypeptide chains

common pool of ribosomal subunits in cytosol

ER membrane

polyribosome

400 nm

Figure 12-41
Protein Translocation Can Be Driven Through Structurally Similar Translocators

No additional energy is needed

An additional complex is attached to the Sec61 translocator and deposits BiP molecules onto the translocating chain as it emerges into the ER lumen. ATP-driven cycles of BiP binding and release pull the protein into the lumen

ATP-hydrolysis-driven conformational changes drive a pistonlike motion in SecA ATPase, each cycle pushing about 20 aa of the protein chain through the pore of the translocator.

Figure 12-44
On binding an ER signal sequence (which acts as a starttransfer signal), the translocator opens its pore, allowing the transfer of the polypeptide chain across the lipid bilayer as a loop. After the protein has been completely translocated, the pore closes, but the translocator now opens laterally within the lipid bilayer, allowing the hydrophobic signal sequence to diffuse into the bilayer, where it is rapidly degraded. * In this figure, the ribosomes have been omitted for clarity.
How a Single-Pass Transmembrane Protein with a Cleaved ER Signal Sequence Is Integrated into the ER Membrane

In this protein, the co-translational translocation process is initiated by an N-terminal ER signal sequence (red) that functions as a start-transfer signal, as in Figure 12–45. In addition to this start-transfer sequence, however, the protein also contains a stop-transfer sequence (orange). When the stop-transfer sequence enters the translocator and interacts with a binding site, the translocator changes its conformation and discharges the protein laterally into the lipid bilayer.

* In this figure, the ribosomes have been omitted for clarity.
An internal ER signal sequence (also binds to the SRP) that functions as start transfer signal can bind to the translocator in one of two different ways, leading to a membrane protein that has either its C-terminus (pathway A) or its N-terminus (pathway B) in the ER lumen.

Proteins are directed into either pathway by **features in the polypeptide chain flanking the internal start transfer sequence.**

* In this figure, the ribosomes have been omitted for clarity.
Integration of a Double-Pass Membrane Protein with an Internal Signal Sequence into the ER Membrane

In this protein, an internal ER signal sequence acts as a start-transfer signal (as in Figure 12–47) and initiates the transfer of the C-terminal part of the protein. At some point after a stop-transfer sequence has entered the translocator, the translocator discharges the sequence laterally into the membrane. * In this figure, the ribosomes have been omitted for clarity.
Figure 12-49

(A) **A hydrophobicity (also called hydropathy) plot** identifies seven short hydrophobic regions in rhodopsin.

(B) The hydrophobic region nearest the N-terminus serves as a start-transfer sequence that causes the preceding N-terminal portion of the protein to pass across the ER membrane. Subsequent hydrophobic sequences function in alternation as start-transfer and stop-transfer sequences.

(C) The final integrated rhodopsin has its N-terminus located in the ER lumen and its C-terminus located in the cytosol. The blue hexagons represent covalently attached oligosaccharides. Arrows indicate the paired start and stop signals inserted into the translocator.
(A) The ClC chloride channel, which consists of two subunits, has a complex arrangement of helices.
(B) The EmrE protein has two identical subunits with completely inverted topology.
(C) The topology of aquaporin 1 changes before and after insertion into the membrane. The red arrows highlight the direction of helix rearrangements. Several helices must either insert into the membrane or flip orientations.
(D) Topology rearrangements in lactose permease with varying lipid composition. The helices that undergo topology rearrangements in the distinct lipid environments are circled in the dashed red box.