ZOO-302:(1.2) Membrane structure and function

Plasma Membrane


The biological membrane, which is present in both eukaryotic and prokaryotic cell. It is also called as cell membrane as it is works as a barrier between the inner and outer surface of a cell. In animal cells, the plasma membrane is present in the outer most layer of the cell and in plant cell it is present just beneath the cell wall.

Structure of Plasma Membrane

Plasma Membrane Definition

Plasma membrane can be defined as a biological membrane or an outer membrane of a cell, which is composed of two layers of phospholipids and embedded with proteins. It is a thin semi permeable membrane layer, which surrounds the cytoplasm and other constituents of the cell.

Function of Plasma Membrane

1. It separates the contents of the cell from its outside environment and it regulates what enters and exits the cell.
2. Plasma membrane plays a vital role in protecting the integrity of the interior of the cell by allowing only selected substances into the cell and keeping other substances out.
3. It also serves as a base of attachment for the cytoskeleton in some organisms and the cell wall in others. Thus the cell membrane supports the cell and helps in maintaining the shape of the cell.
4. The cell membrane is primarily composed of proteins and lipids. While lipids help to give membranes their flexibility and proteins monitor and maintain the cell's chemical climate and assist in the transfer of molecules across the membrane.
5. The lipid bilayer is semi-permeable, which allows only selected molecules to diffuse across the membrane.
Characteristics of Plasma Membrane

Below you could see characteristics of plasma membrane

1. The plasma membrane (cell membrane) is made of two layers of phospholipids.
2. The plasma membrane has many proteins embedded in it.
3. The plasma membrane regulates the entry and exit of the cell. Many molecules cross the cell membrane by diffusion and osmosis.
4. The fundamental structure of the membrane is phospholipid bilayer and it forms a stable barrier between two aqueous compartments.
5. The proteins present in the plasma membrane, act as pumps, channels, receptors, enzymes or structural components.

Plasma Membrane Structure

1. It is the boundary, which separates the living cell from their non-living surroundings.
2. It is the phospholipids bilayer.
3. Plasma membrane is an amphipathic, which contains both hydrophilic heads and hydrophobic tails.
4. It is a fluid mosaic of lipids, proteins and carbohydrate.
5. It is lipid bilayer, which contains -two layers of phospholipids, phosphate head is polar (water loving), fatty acid tails non-polar (water fearing) and the proteins embedded in membrane.

Components of Plasma Membrane

The main components of plasma membrane include:

1. Proteins like glycoprotein, which are used for cell recognition and act as receptors and antigens.
2. Proteins like glycolipids are attached to phospholipids along with the sugar chains.
3. Lipids with short chain of carbohydrates are attached on the extracellular side of the membrane.
4. Phospholipid Bilayer - which are made up of phosphates and lipids. They create a partially permeable membrane, which allows only certain substances to diffuse through the membrane.
5. Cholesterol – it maintains the fluidity of cell surface membrane.

**Components of Plasma Membrane**

![Plasma Membrane Structure](image)

**Proteins in Plasma Membrane**
In plasma membrane, a protein helps in providing the support and shape to the cell. There are three types of proteins in plasma membrane, which includes:

1. **Cell membrane receptor proteins** - It helps in communication of a cell with their external environment with the help of hormones, neurotransmitters and other signaling molecules.
2. **Transport proteins** - It helps in transporting molecules across cell membranes through facilitated diffusion. For example: globular proteins.
3. **Glycoprotein** - It helps in cell to cell communications and molecule transport across the membrane.

**Prokaryotic Plasma Membrane**
The prokaryotic plasma membranes are composed of phospholipids bilayer with embedded proteins. In the middle of the bilayer, the fatty acids of the phospholipids are found, which is called as hydrophobic region. Prokaryotic cells can have multiple plasma membranes. In prokaryotic organisms, plasma membranes are responsible for controlling the entry and exit of the cell.

**Eukaryotic Plasma Membrane**
The eukaryotic plasma membrane is a phospholipids bilayer containing proteins and carbohydrates attached to the proteins and sterols. It is a fluid phospholipids bilayer embedded with proteins and
glycoprotein. The phospholipids bilayer is arranged in such a manner that they form the center of the membrane. They also contain sterols, which makes the membrane less permeable and helps to stabilize the membrane and add the rigidity to membranes.

**Structure of the Plasma Membrane**


(https://www.ncbi.nlm.nih.gov/books/NBK9898/)

Like all other cellular membranes, the plasma membrane consists of both lipids and proteins. The fundamental structure of the membrane is the phospholipid bilayer, which forms a stable barrier between two aqueous compartments. In the case of the plasma membrane, these compartments are the inside and the outside of the cell. Proteins embedded within the phospholipid bilayer carry out the specific functions of the plasma membrane, including selective transport of molecules and cell-cell recognition.

**The Phospholipid Bilayer**

The plasma membrane is the most thoroughly studied of all cell membranes, and it is largely through investigations of the plasma membrane that our current concepts of membrane structure have evolved. The plasma membranes of mammalian red blood cells (erythrocytes) have been particularly useful as a model for studies of membrane structure. Mammalian red blood cells do not contain nuclei or internal membranes, so they represent a source from which pure plasma membranes can be easily isolated for biochemical analysis. Indeed, studies of the red blood cell plasma membrane provided the first evidence that biological membranes consist of lipid bilayers. In 1925, two Dutch scientists (E. Gorter and R. Grendel) extracted the membrane lipids from a known number of red blood cells, corresponding to a known surface area of plasma membrane. They then determined the surface area occupied by a monolayer of the extracted lipid spread out at an air-water interface. The surface area of the lipid monolayer turned out to be twice that occupied by the erythrocyte plasma membranes, leading to the conclusion that the membranes consisted of lipid bilayers rather than monolayers.

The bilayer structure of the erythrocyte plasma membrane is clearly evident in high-magnification electron micrographs (Figure 12.1). The plasma membrane appears as two dense lines separated by an intervening space—a morphology frequently referred to as a “railroad track” appearance. This image results from the binding of the electron-dense heavy metals used as stains in transmission electron microscopy (see Chapter 1) to the polar head groups of the phospholipids, which therefore appear as dark lines. These dense lines are separated by the lightly stained interior portion of the membrane, which contains the hydrophobic fatty acid chains.

As discussed in Chapter 2, the plasma membranes of animal cells contain four major phospholipids (phosphatidylcholine, phosphatidylethanolamine, phosphatidylserine, and sphingomyelin), which together account for more than half of the lipid in most membranes. These phospholipids are asymmetrically distributed between the two halves of the membrane bilayer (Figure 12.2). The outer leaflet of the plasma membrane consists mainly of phosphatidylcholine and sphingomyelin, whereas phosphatidylethanolamine and...
phosphatidylserine are the predominant phospholipids of the inner leaflet. A fifth phospholipid, **phosphatidylinositol**, is also localized to the inner half of the plasma membrane. Although phosphatidylinositol is a quantitatively minor membrane component, it plays an important role in cell signaling, as discussed in the next chapter. The head groups of both phosphatidylserine and phosphatidylinositol are negatively charged, so their predominance in the inner leaflet results in a net negative charge on the cytosolic face of the plasma membrane.

**Figure 12.2**

Lipid components of the plasma membrane. The outer leaflet consists predominantly of phosphatidylcholine, sphingomyelin, and glycolipids, whereas the inner leaflet contains phosphatidylethanolamine, phosphatidylserine, and phosphatidylinositol. Cholesterol (more...)

In addition to the **phospholipids**, the plasma membranes of animal cells contain **glycolipids** and **cholesterol**. The glycolipids are found exclusively in the outer leaflet of the **plasma membrane**, with their **carbohydrate** portions exposed on the cell surface. They are relatively minor membrane components, constituting only about 2% of the **lipids** of most plasma membranes. Cholesterol, on the other hand, is a major membrane constituent of animal cells, being present in about the same molar amounts as the phospholipids.

Two general features of phospholipid bilayers are critical to membrane function. First, the structure of **phospholipids** is responsible for the basic function of membranes as barriers between two aqueous compartments. Because the interior of the **phospholipid bilayer** is occupied by **hydrophobic** fatty acid chains, the membrane is impermeable to water-soluble molecules, including ions and most biological molecules. Second, bilayers of the naturally occurring phospholipids are viscous fluids, not solids. The **fatty acids** of most natural phospholipids have one or more double bonds, which introduce kinks into the hydrocarbon chains and make them difficult to pack together. The long hydrocarbon chains of the fatty acids therefore move freely in the interior of the membrane, so the membrane itself is soft and flexible. In addition, both phospholipids and **proteins** are free to diffuse laterally within the membrane—a property that is critical for many membrane functions.

Because of its rigid ring structure, **cholesterol** plays a distinct role in membrane structure. Cholesterol will not form a membrane by itself, but inserts into a bilayer of **phospholipids** with its polar hydroxyl group close to the phospholipid head groups (see **Figure 12.2**). Depending on the temperature, cholesterol has distinct effects on membrane fluidity. At high temperatures, cholesterol interferes with the movement of the phospholipid fatty acid chains, making the outer
part of the membrane less fluid and reducing its permeability to small molecules. At low temperatures, however, cholesterol has the opposite effect: By interfering with interactions between fatty acid chains, cholesterol prevents membranes from freezing and maintains membrane fluidity. Although cholesterol is not present in bacteria, it is an essential component of animal cell plasma membranes. Plant cells also lack cholesterol, but they contain related compounds (sterols) that fulfill a similar function.

Recent studies suggest that not all lipids diffuse freely in the plasma membrane. Instead, discrete membrane domains appear to be enriched in cholesterol and the sphingolipids (sphingomyelin and glycolipids). These clusters of sphingolipids and cholesterol are thought to form “rafts” that move laterally within the plasma membrane and may associate with specific membrane proteins. Although the functions of lipid rafts remain to be understood, they may play important roles in processes such as cell signaling and the uptake of extracellular molecules by endocytosis.

Go to:

**Membrane Proteins**

While lipids are the fundamental structural elements of membranes, proteins are responsible for carrying out specific membrane functions. Most plasma membranes consist of approximately 50% lipid and 50% protein by weight, with the carbohydrate portions of glycolipids and glycoproteins constituting 5 to 10% of the membrane mass. Since proteins are much larger than lipids, this percentage corresponds to about one protein molecule per every 50 to 100 molecules of lipid. In 1972, Jonathan Singer and Garth Nicolson proposed the fluid mosaic model of membrane structure, which is now generally accepted as the basic paradigm for the organization of all biological membranes. In this model, membranes are viewed as two-dimensional fluids in which proteins are inserted into lipid bilayers (Figure 12.3).

![Figure 12.3](image)

**Figure 12.3**

Fluid mosaic model of the plasma membrane. Integral membrane proteins are inserted into the lipid bilayer, whereas peripheral proteins are bound to the membrane indirectly by protein-protein interactions. Most integral membrane proteins are transmembrane (more...)
Singer and Nicolson distinguished two classes of membrane-associated proteins, which they called **peripheral** and **integral membrane proteins**. Peripheral membrane proteins were operationally defined as proteins that dissociate from the membrane following treatments with polar reagents, such as solutions of extreme pH or high salt concentration, that do not disrupt the **phospholipid bilayer**. Once dissociated from the membrane, **peripheral membrane proteins** are soluble in aqueous buffers. These proteins are not inserted into the **hydrophobic** interior of the lipid bilayer. Instead, they are indirectly associated with membranes through protein-protein interactions. These interactions frequently involve ionic bonds, which are disrupted by extreme pH or high salt.

In contrast to the **peripheral membrane proteins**, **integral membrane proteins** can be released only by treatments that disrupt the **phospholipid bilayer**. Portions of these integral membrane proteins are inserted into the lipid bilayer, so they can be dissociated only by reagents that disrupt **hydrophobic** interactions. The most commonly used reagents for solubilization of integral membrane proteins are detergents, which are small **amphipathic** molecules containing both hydrophobic and hydrophilic groups. The hydrophobic portions of detergents displace the membrane **lipids** and bind to the hydrophobic portions of integral membrane proteins. Because the other end of the detergent molecule is hydrophilic, the detergent-protein complexes are soluble in aqueous solutions.

**Figure 12.4**

Solubilization of integral membrane proteins by detergents. Detergents (e.g., octyl glucoside) are amphipathic molecules containing hydrophilic head groups and hydrophobic tails. The hydrophobic tails bind to the hydrophobic regions of integral membrane proteins.

Many integral proteins are **transmembrane proteins**, which span the lipid bilayer with portions exposed on both sides of the membrane. These proteins can be visualized in electron micrographs of plasma membranes prepared by the freeze-fracture technique. In these specimens, the membrane is split and separates into its two leaflets. Transmembrane proteins are then apparent as particles on the internal faces of the membrane.

**Figure 12.5**

Freeze-fracture electron micrograph of human red blood cell membranes. The particles in the membrane are transmembrane proteins.

The membrane-spanning portions of **transmembrane proteins** are usually α helices of 20 to 25 hydrophobic amino acids that are inserted into the membrane of the **endoplasmic reticulum** during synthesis of the **polypeptide chain**. These proteins are then transported in membrane vesicles from the endoplasmic reticulum to the **Golgi apparatus**, and from there to the **plasma membrane**. Carbohydrate groups are added to the polypeptide chains in both the endoplasmic reticulum and Golgi apparatus, so most...
transmembrane proteins of the plasma membrane are glycoproteins with their oligosaccharides exposed on the surface of the cell.

Studies of red blood cells have provided good examples of both peripheral and integral proteins associated with the plasma membrane. The membranes of human erythrocytes contain about a dozen major proteins, which were originally identified by gel electrophoresis of membrane preparations. Most of these are peripheral membrane proteins that have been identified as components of the cortical cytoskeleton, which underlies the plasma membrane and determines cell shape (see Chapter 11). For example, the most abundant peripheral membrane protein of red blood cells is spectrin, which is the major cytoskeletal protein of erythrocytes. Other peripheral membrane proteins of red blood cells include actin, ankyrin, and band 4.1. Ankyrin serves as the principal link between the plasma membrane and the cytoskeleton by binding to both spectrin and the integral membrane protein band 3 (see Figure 11.11). An additional link between the membrane and the cytoskeleton is provided by band 4.1, which binds to the junctions of spectrin and actin, as well as to glycophorin (the other major integral membrane protein of erythrocytes).

The two major integral membrane proteins of red blood cells, glycophorin and band 3, provide well-studied examples of transmembrane protein structure (Figure 12.6). Glycophorin is a small glycoprotein of 131 amino acids, with a molecular weight of about 30,000, half of which is protein and half carbohydrate. Glycophorin crosses the membrane with a single membrane-spanning α helix of 23 amino acids, with its glycosylated amino-terminal portion exposed on the cell surface. Although glycophorin was one of the first transmembrane proteins to be characterized, its precise function remains unknown. In contrast, the function of the other major transmembrane protein of red blood cells is well understood. This protein, originally known as band 3, is the anion transporter responsible for the passage of bicarbonate (HCO₃⁻) and chloride (Cl⁻) ions across the red blood cell membrane. The band 3 polypeptide chain is 929 amino acids and is thought to have 14 membrane-spanning α-helical regions. Within the membrane, dimers of band 3 form globular structures containing internal channels through which ions are able to travel across the lipid bilayer.

![Figure 12.6](image)

Integral membrane proteins of red blood cells. Glycophorin (131 amino acids) contains a single transmembrane α helix. It is heavily glycosylated, with oligosaccharides attached to 16 sites on the extracellular portion of the polypeptide chain. (more...)

Because of their amphipathic character, transmembrane proteins have proved difficult to crystallize, as required for three-dimensional structural analysis by X-ray diffraction. The first
transmembrane protein to be analyzed by X-ray crystallography was the photosynthetic reaction center of the bacterium *Rhodopseudomonas viridis*, whose structure was reported in 1985 (Figure 12.7). The reaction center contains three transmembrane proteins, designated L, M, and H (light, medium, and heavy) according to their apparent sizes indicated by gel electrophoresis. The L and M subunits each have five membrane-spanning α helices. The H subunit has only a single transmembrane α helix, with the bulk of the polypeptide chain on the cytosolic side of the membrane. The fourth subunit of the reaction center is a cytochrome, which is a peripheral membrane protein bound to the complex by protein-protein interactions.

![Figure 12.7](image)

**Figure 12.7**

A bacterial photosynthetic reaction center. The reaction center consists of three transmembrane proteins, designated L (red), M (yellow), and H (green). The L and M subunits each have five transmembrane α helices, whereas the H subunit has only (more...)

Although most transmembrane proteins span the membrane by α-helical regions, this is not always the case. A well-characterized exception is provided by the porins—a class of proteins that form channels in the outer membranes of some bacteria. Many bacteria, including *E. coli*, have a dual membrane system in which the plasma membrane (or inner membrane) is surrounded by the cell wall and a distinct outer membrane (Figure 12.8). In contrast to the plasma membrane, the outer membrane is highly permeable to ions and small polar molecules (in the case of *E. coli*, with molecular weights up to 600). This permeability results from the porins, which form open aqueous channels through the lipid bilayer. As discussed in Chapter 10, proteins related to the bacterial porins are also found in the outer membranes of mitochondria and chloroplasts.

![Figure 12.8](image)

**Figure 12.8**

Bacterial outer membranes. The plasma membrane of some bacteria is surrounded by a cell wall and a distinct outer membrane. The outer membrane contains porins, which form open aqueous channels allowing the free passage of ions and small molecules.

Structural analysis has indicated that the porins do not contain hydrophobic α-helical regions. Instead, they cross the membrane as β barrels, in which 16 β sheets fold up into a barrel-like structure enclosing an aqueous pore (Figure 12.9). The side chains of polar amino acids line the pore, whereas side chains of hydrophobic amino acids interact with the interior of the membrane. The porin monomers associate to form stable trimers, each of which contains three open channels through which polar molecules can diffuse across the membrane.
In contrast to transmembrane proteins, a variety of proteins (many of which behave as integral membrane proteins) are anchored in the plasma membrane by covalently attached lipids or glycolipids (Figure 12.10). Members of one class of these proteins are inserted into the outer leaflet of the plasma membrane by glycosylphosphatidylinositol (GPI) anchors. GPI anchors are added to certain proteins that have been transferred into the endoplasmic reticulum and are anchored in the membrane by a C-terminal transmembrane region (see Figure 9.16). The transmembrane region is cleaved as the GPI anchor is added, so these proteins remain attached to the membrane only by the glycolipid. Since the polypeptide chains of GPI-anchored proteins are transferred into the endoplasmic reticulum, they are glycosylated and exposed on the surface of the cell following transport to the plasma membrane.

Other proteins are anchored in the inner leaflet of the plasma membrane by covalently attached lipids. Rather than being processed through the secretory pathway, these proteins are synthesized on free cytosolic ribosomes and then modified by the addition of lipids. These modifications include the addition of myristic acid (a 14-carbon fatty acid) to the amino terminus of the polypeptide chain, the addition of palmitic acid (16 carbons) to the side chains of cysteine residues, and the addition of prenyl groups (15 or 20 carbons) to the side chains of carboxy-terminal cysteine residues (see Figures 7.29, 7.30, and 7.31). In some cases, these proteins (many of which behave as peripheral membrane proteins) are targeted to the plasma membrane by positively charged regions of the polypeptide chain as well as by the attached lipids. These positively charged protein domains may interact with the negatively charged head groups of phosphatidylserine on the cytosolic face of the plasma membrane. It is noteworthy that many of the proteins anchored in the inner leaflet of the plasma membrane (including the Src and Ras proteins illustrated in Figure 12.10) play important roles in the transmission of signals from cell surface receptors to intracellular targets, as discussed in the next chapter.
Mobility of Membrane Proteins

Membrane proteins and phospholipids are unable to move back and forth between the inner and outer leaflets of the membrane at an appreciable rate. However, because they are inserted into a fluid lipid bilayer, both proteins and lipids are able to diffuse laterally through the membrane. This lateral movement was first shown directly in an experiment reported by Larry Frye and Michael Edidin in 1970, which provided support for the fluid mosaic model. Frye and Edidin fused human and mouse cells in culture to produce human-mouse cell hybrids (Figure 12.11). They then analyzed the distribution of proteins in the membranes of these hybrid cells using antibodies that specifically recognize proteins of human and mouse origin. These antibodies were labeled with different fluorescent dyes, so the human and mouse proteins could be distinguished by fluorescence microscopy. Immediately after fusion, human and mouse proteins were localized to different halves of the hybrid cells. However, after a brief period of incubation at 37°C, the human and mouse proteins were completely intermixed over the cell surface, indicating that they moved freely through the plasma membrane.

![Figure 12.11](image)

**Figure 12.11**

Mobility of membrane proteins. Human and mouse cells were fused to produce hybrid cells. The distribution of cell surface proteins was then analyzed using anti-human and anti-mouse antibodies labeled with different fluorescent dyes (red and green, respectively). (more...)

However, not all proteins are able to diffuse freely through the membrane. In some cases, the mobility of membrane proteins is restricted by their association with the cytoskeleton. For example, a fraction of band 3 in the red blood cell membrane is immobilized as a result of its association with ankyrin and spectrin. In other cases, the mobility of membrane proteins may be restricted by their associations with other membrane proteins, with proteins on the surface of adjacent cells, or with the extracellular matrix.

In contrast to blood cells, epithelial cells are polarized when they are organized into tissues, with different parts of the cell responsible for performing distinct functions. Consequently, the plasma membranes of many epithelial cells are divided into distinct apical and basolateral domains that differ in function and protein composition (Figure 12.12). For example, epithelial cells of the small intestine function to absorb nutrients from the digestive tract. The apical surface of these cells, which faces the intestinal lumen, is therefore covered by microvilli and specialized for nutrient absorption. The basolateral surface, which faces underlying connective tissue and the blood supply, is specialized to mediate the transfer of absorbed nutrients into the circulation. In order to maintain these distinct functions, the mobility of plasma membrane proteins must be
restricted to the appropriate domains of the cell surface. At least part of the mechanism by which this occurs involves the formation of tight junctions (which are discussed later in this chapter) between adjacent cells of the epithelium. These junctions not only seal the space between cells but also serve as barriers to the movement of membrane lipids and proteins. As a result, proteins are able to diffuse within either the apical or basolateral domains of the plasma membrane but are not able to cross from one domain to the other.

**Figure 12.12**

A polarized intestinal epithelial cell. The apical surface of the cell contains microvilli and is specialized for absorption of nutrients from the intestinal lumen. The basolateral surface is specialized for the transfer of absorbed nutrients to the underlying (more...)

The Glycocalyx

As already discussed, the extracellular portions of plasma membrane proteins are generally glycosylated. Likewise, the carbohydrate portions of glycolipids are exposed on the outer face of the plasma membrane. Consequently, the surface of the cell is covered by a carbohydrate coat, known as the glycocalyx, formed by the oligosaccharides of glycolipids and transmembrane glycoproteins (Figure 12.13).

**Figure 12.13**

The glycocalyx. An electron micrograph of intestinal epithelium illustrating the glycocalyx (arrows). (Don Fawcett/Visuals Unlimited.)

Part of the role of the glycocalyx is to protect the cell surface. In addition, the oligosaccharides of the glycocalyx serve as markers for a variety of cell-cell interactions. A well-studied example of these interactions is the adhesion of white blood cells (leukocytes) to the endothelial cells that line blood vessels—a process that allows the leukocytes to leave the circulatory system and mediate the inflammatory response in injured tissues. The initial step in adhesion between leukocytes and endothelial cells is mediated by a family of transmembrane proteins called selectins, which recognize specific carbohydrates on the cell surface (Figure 12.14). Two members of the selectin family (E-selectin and P-selectin), expressed by endothelial cells and platelets, bind to specific oligosaccharides expressed on the surface of leukocytes. A
different selectin (L-selectin) is expressed by leukocytes and recognizes an oligosaccharide on the surface of endothelial cells. The oligosaccharides exposed on the cell surface thus provide a set of markers that help identify the distinct cell types of multicellular organisms.

![Figure 12.14](https://www.khanacademy.org/science/biology/membranes-and-transport/active-transport/a/active-transport)

**Figure 12.14**

Binding of selectins to oligosaccharides. E-selectin is a transmembrane protein expressed by endothelial cells that binds to an oligosaccharide expressed on the surface of leukocytes. The oligosaccharide recognized by E-selectin contains N-acetylglucosamine (more...)

**Active transport**


**Introduction**

Passive transport is a great strategy for moving molecules into or out of a cell. It's cheap, it's easy, and all the cell has to do is sit there and let the molecules diffuse in. But...it also doesn't work in every situation. For instance, suppose the sugar glucose is more concentrated inside of a cell than outside. If the cell needs more sugar in to meet its metabolic needs, how can it get that sugar in?

Here, the cell can't import glucose for free using diffusion, because the natural tendency of the glucose will be to diffuse *out* rather than flowing *in*. Instead, the cell must bring in more glucose molecules via **active transport**. In active transport, unlike passive transport, the cell expends energy (for example, in the form of ATP) to move a substance against its concentration gradient.

Here, we’ll look in more detail at gradients of molecules that exist across cell membranes, how they can help or hinder transport, and how active transport mechanisms allow molecules to move against their gradients.

**Electrochemical gradients**

We have already discussed simple concentration gradients, in which a substance is found in different concentrations over a region of space or on opposite sides of a membrane. However, because atoms and molecules can form ions and carry positive or negative electrical charges, there may also be an electrical gradient, or difference in charge, across a plasma membrane. In
fact, living cells typically have what’s called a **membrane potential**, an electrical potential difference (voltage) across their cell membrane.

Image depicting the charge and ion distribution across the membrane of a typical cell. Overall, there are more positive charges on the outside of the membrane than on the inside. The concentration of sodium ions is lower inside the cell than in the extracellular fluid, while the reverse is true for potassium ions.

*Image credit: image from OpenStax Biology, originally by Synaptitude/Wikimedia Commons.*

An electrical potential difference exists whenever there is a net separation of charges in space. In the case of a cell, positive and negative charges are separated by the barrier of the cell membrane, with the inside of the cell having extra negative charges relative to the outside. The membrane potential of a typical cell is -40 to -80 millivolts, with the minus sign meaning that inside of the cell is more negative than the outside. The cell actively maintains this membrane potential, and we’ll see how it forms in the section on the sodium-potassium pump (below).

As an example of how the membrane potential can affect ion movement, let’s look at sodium and potassium ions. In general, the inside of a cell has a higher concentration of potassium (K^+ start superscript, plus, end superscript) and a lower concentration of sodium (Na^+ start superscript, plus, end superscript) than the extracellular fluid around it.

- If sodium ions are outside of a cell, they will tend to move into the cell based on both their concentration gradient (the lower concentration of Na^+ start superscript, plus, end superscript in the cell) and the voltage across the membrane (the more negative charge on the inside of the membrane).
- Because K^+ start superscript, plus, end superscript is positive, the voltage across the membrane will encourage its movement into the cell, but its concentration gradient will tend to drive it out of the cell (towards the region of lower concentration). The final concentrations of potassium on the two sides of the membrane will be a balance between these opposing forces. The combination of concentration gradient and voltage that affects an ion’s movement is called the **electrochemical gradient**.

**Active transport: moving against a gradient**

To move substances against a concentration or electrochemical gradient, a cell must use energy. Active transport mechanisms do just this, expending energy (often in the form of ATP) to maintain the right concentrations of ions and molecules in living cells. In fact, cells spend much
of the energy they harvest in metabolism to keep their active transport processes running. For instance, most of a red blood cell’s energy is used to maintain internal sodium and potassium levels that differ from those of the surrounding environment.

Active transport mechanisms can be divided into two categories. **Primary active transport** directly uses a source of chemical energy (e.g., ATP) to move molecules across a membrane against their gradient. **Secondary active transport (cotransport)**, on the other hand, uses an electrochemical gradient – generated by active transport – as an energy source to move molecules against their gradient, and thus does not directly require a chemical source of energy such as ATP. We’ll look at each type of active transport in greater detail below.

**Primary active transport**

One of the most important pumps in animal cells is the **sodium-potassium pump**, which moves Na^{+} plus, end superscript out of cells, and K^{+} into them. Because the transport process uses ATP as an energy source, it is considered an example of primary active transport.

Not only does the sodium-potassium pump maintain correct concentrations of Na^{+} and K^{+}, but it also plays a major role in generating the voltage across the cell membrane in animal cells. Pumps like this, which are involved in the establishment and maintenance of membrane voltages, are known as **electrogenic pumps**. The primary electrogenic pump in plants is one that pumps hydrogen ions (H^{+}, rather than sodium and potassium) across the membrane.

**The sodium-potassium pump cycle**

![Figure showing the transport cycle of the sodium-potassium pump.](https://www.openstax.org/details/html5 tabIndex=0)

*Image credit: OpenStax Biology. Image modified from original work by Mariana Ruiz Villareal.*

The sodium-potassium pump transports sodium out of and potassium into the cell in a repeating cycle of conformational (shape) changes. In each cycle, three sodium ions exit the cell, while two potassium ions enter. This process takes place in the following steps:

1. To begin, the pump is open to the inside of the cell. In this form, the pump really likes to bind (has a high affinity for) sodium ions, and will take up three of them.
2. When the sodium ions bind, they trigger the pump to hydrolyze (break down) ATP. One phosphate group from ATP is attached to the pump, which is then said to be phosphorylated. ADP is released as a by-product.

3. Phosphorylation makes the pump change shape, re-orienting itself so it opens towards the extracellular space. In this conformation, the pump no longer likes to bind to sodium ions (has a low affinity for them), so the three sodium ions are released outside the cell.

4. In its outward-facing form, the pump switches allegiances and now really likes to bind to (has a high affinity for) potassium ions. It will bind two of them, and this triggers removal of the phosphate group attached to the pump in step 2.

5. With the phosphate group gone, the pump will change back to its original form, opening towards the interior of the cell.

6. In its inward-facing shape, the pump loses its interest in (has a low affinity for) potassium ions, so the two potassium ions will be released into the cytoplasm. The pump is now back to where it was in step 1, and the cycle can begin again.

This may seem like a complicated cycle, but it just involves the protein going back and forth between two forms: an inward-facing form with high affinity for sodium (and low affinity for potassium), and an outward-facing form with high affinity for potassium (and low affinity for sodium). The protein can be toggled back and forth between these forms by the addition or removal of a phosphate group, which is in turn controlled by the binding of the ions to be transported.

**How the sodium-potassium pump generates a membrane potential**

How, exactly, does the sodium-potassium pump establish a voltage across the membrane? It’s tempting to simply make an argument based on stoichiometry: for every three ions of sodium that move out, only two ions of potassium move in, resulting in a more negative cell interior. While this charge ratio does make the cell’s interior slightly more negative, it actually accounts for only a tiny fraction of the sodium-potassium pump’s effect on membrane potential.

Instead, the sodium-potassium pump acts primarily by building up a high concentration of potassium ions inside the cell, which makes potassium’s concentration gradient very steep. The gradient is steep enough that potassium ions will move out of the cell (via channels), despite a growing negative charge on the interior. This process continues until the voltage across the membrane is large enough to counterbalance potassium’s concentration gradient. At this balance point, the inside of the membrane is negative relative to the outside. This voltage will be maintained as long as $\text{K}^{\text{++}}$ is imported, and the concentration in the cell stays high, but will disappear if $\text{K}^{\text{++}}$ is released.

For more explanation of how the voltage across the membrane is established, take a look at the [membrane potential](#) article in the neurobiology section.

**Secondary active transport**

The electrochemical gradients set up by primary active transport store energy, which can be released as the ions move back down their gradients. Secondary active transport uses the energy stored in these gradients to move other substances against their own gradients.

As an example, let's suppose we have a high concentration of sodium ions in the extracellular space (thanks to the hard work of the sodium-potassium pump). If a route such as a channel or
carrier protein is open, sodium ions will move down their concentration gradient and return to the interior of the cell.

In secondary active transport, the movement of the sodium ions down their gradient is coupled to the uphill transport of other substances by a shared carrier protein (a **cotransporter**). For instance, in the figure below, a carrier protein lets sodium ions move down their gradient, but simultaneously brings a glucose molecule up its gradient and into the cell. The carrier protein uses the energy of the sodium gradient to drive the transport of glucose molecules.

Diagram of a sodium-glucose cotransporter, which uses the energy stored in a sodium ion gradient to transport glucose "uphill" against its gradient. The cotransporter accomplishes this by physically coupling the transport of glucose to the movement of sodium ions down their concentration gradient.

*Image modified from "Active transport: Figure 4," by OpenStax College, Biology (CC BY 3.0) and "Scheme secondary transport," by Mariana Ruiz Villareal (public domain).*

In secondary active transport, the two molecules being transported may move either in the same direction (i.e., both into the cell), or in opposite directions (i.e., one into and one out of the cell). When they move in the same direction, the protein that transports them is called a **symporter**, while if they move in opposite directions, the protein is called an **antiporter**.
Simple diagram of a symporter (carrying two molecules in the same direction) and an antiporter (carrying two molecules in opposite directions).

*Image modified from OpenStax Biology. Original image by Lupask/Wikimedia Commons.*

**ION PUMPS**

**Active Transport by ATP-Powered Pumps**

*Molecular Cell Biology. 4th edition.*

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We turn now to the ATP-powered pumps that transport ions and various small molecules against their concentration gradients. The general structures of the four principal classes of these transport proteins are depicted in Figure 15-10, and their properties are summarized in Table 15-2. Note that the P, F, and V classes transport ions only, whereas the ABC superfamily class transports small molecules as well as ions.

*Figure 15-10*

The four classes of ATP-powered transport proteins. P-class pumps are composed of two different polypeptides, α and β, and become phosphorylated as part of the transport cycle. The sequence around the phosphorylated residue, located in (more...)
**Table 15-2**

Comparison of Major Classes of ATP-Powered Ion and Small-Molecule Pumps.

*P-class ion pumps* contain a transmembrane catalytic α subunit, which contains an ATP-binding site, and usually a smaller β subunit, which may have regulatory functions. Many of these pumps are tetramers composed of two α and two β subunits. During the transport process, at least one of the α subunits is phosphorylated (hence the label “P”), and the transported ions are thought to move through the phosphorylated subunit. This class includes the Na⁺/K⁺ ATPase in the plasma membrane, which maintains the Na⁺ and K⁺ gradients typical of animal cells, and several Ca²⁺ATPases, which pump Ca²⁺ ions out of the cytosol into the external medium or into the lumen of the sarcoplasmic reticulum (SR) of muscle cells. Another member of the P class, found in acid-secreting cells of the mammalian stomach, transports protons (H⁺ ions) out of and K⁺ ions into the cell. The H⁺ pump that maintains the membrane electric potential in plant, fungal, and bacterial cells also belongs to this class.

The structures of *F-class* and *V-class ion pumps* are similar to each other but unrelated to and more complicated than P-class pumps. F- and V-class pumps contain at least three kinds of transmembrane proteins and five kinds of extrinsic polypeptides that form the cytosolic domain. Several of the transmembrane and extrinsic subunits in F-class and V-class pumps exhibit sequence homology, and each pair of homologous subunits is thought to have evolved from a common polypeptide.

All known V and F pumps transport only protons in a process that does not involve a phosphoprotein intermediate. V-class pumps generally function to maintain the low pH of plant vacuoles and of lysosomes and other acidic vesicles in animal cells by using the energy released by ATP hydrolysis to pump protons from the cytosolic to the exoplasmic face of the membrane against the proton electrochemical gradient. F-class pumps are found in bacterial plasma membranes and in mitochondria and chloroplasts. In contrast to V pumps, they generally function to power the synthesis of ATP from ADP and P, by movement of protons from the exoplasmic to the cytosolic face of the membrane down the proton electrochemical gradient. Because of their importance in ATP synthesis in chloroplasts and mitochondria, F-class proton pumps are treated separately in the next chapter.

The final class of ATP-powered transport proteins is larger and more diverse than the other classes. Referred to as the *ABC (ATP-binding cassette) superfamily*, this class includes more than 100 different transport proteins found in organisms ranging from bacteria to humans. Each ABC protein is specific for a single substrate or group of related substrates including ions, sugars, peptides, polysaccharides, and even proteins. All ABC transport proteins share a common organization consisting of four “core” domains: two transmembrane (T) domains, forming the
passageway through which transported molecules cross the membrane, and two cytosolic ATP-binding (A) domains. In some ABC proteins, the core domains are present in four separate polypeptides; in others, the core domains are fused into one or two multidomain polypeptides.

All classes of ATP-powered pumps have one or more binding sites for ATP, and these are always on the cytosolic face of the membrane (see Figure 15-10). Although these proteins are often called ATPases, they normally do not hydrolyze ATP into ADP and P, unless ions or other molecules are simultaneously transported. Because of the tight coupling between ATP hydrolysis and transport, the energy stored in the phosphoanhydride bond is not dissipated. Thus ATP-powered transport proteins are able to collect the free energy released during ATP hydrolysis and use it to move ions or other molecules uphill against a potential or concentration gradient.

The energy expended by cells to maintain the concentration gradients of Na⁺, K⁺, H⁺, and Ca²⁺ across the plasma and intracellular membranes is considerable. In nerve and kidney cells, for example, up to 25 percent of the ATP produced by the cell is used for ion transport; in human erythrocytes, up to 50 percent of the available ATP is used for this purpose. In cells treated with poisons that inhibit the aerobic production of ATP (e.g., 2,4-dinitrophenol), the ion concentration inside the cell gradually approaches that of the exterior environment as the ions move through plasma membrane channels down their electric and concentration gradients. Eventually treated cells die: partly because protein synthesis requires a high concentration of K⁺ ions and partly because in the absence of a Na⁺ gradient across the cell membrane, a cell cannot import certain nutrients such as amino acids. Studies on the effects of such poisons provided early evidence for the existence of ion pumps. In this section, we discuss in some detail examples of the P, V, and ABC classes of ATP-powered pumps.

**Plasma-Membrane Ca²⁺ ATPase Exports Ca²⁺ Ions from Cells**

As discussed in Chapter 20, small increases in the concentration of free Ca²⁺ ions in the cytosol trigger a variety of cellular responses. In order for Ca²⁺ to function in intracellular signaling, its cytosolic concentration usually must be kept below 0.1 – 0.2 μM. (Although some cytosolic Ca²⁺ is bound to negatively charged groups, it is the concentration of free, unbound Ca²⁺ that is critical to its signaling function.) The plasma membranes of animal, yeast, and probably plant cells contain Ca²⁺-ATPases that transport Ca²⁺ out of the cell against its electrochemical gradient. These P-class ion pumps help maintain the concentration of free Ca²⁺ ions in the cytosol at a low level.

In addition to a catalytic α subunit containing an ATP-binding site, as found in other P-class pumps, plasmamembrane Ca²⁺ ATPases also contain the Ca²⁺-binding regulatory protein calmodulin. A rise in cytosolic Ca²⁺ induces the binding of Ca²⁺ ions to calmodulin, which triggers an allosteric activation of the Ca²⁺ ATPase; as a result, the export of Ca²⁺ ions from the cell accelerates, and the original low cytosolic concentration of free Ca²⁺ is restored rapidly.

**Muscle Ca²⁺ ATPase Pumps Ca²⁺ Ions from the Cytosol into the Sarcoplasmic Reticulum**
Besides the plasma-membrane Ca\(^{2+}\) ATPase, muscle cells contain a second, different Ca\(^{2+}\) ATPase that transports Ca\(^{2+}\) from the cytosol into the lumen of the sarcoplasmic reticulum (SR), an internal organelle that concentrates and stores Ca\(^{2+}\) ions. As discussed in Chapter 18, the SR and its calcium pump (referred to as the muscle calcium pump) are critical in muscle contraction and relaxation: release of Ca\(^{2+}\) ions from the SR into the muscle cytosol causes contraction, and the rapid removal of Ca\(^{2+}\) ions from the cytosol by the muscle calcium pump induces relaxation.

Because the muscle calcium pump constitutes more than 80 percent of the integral protein in SR membranes, it is easily purified and characterized. Each transmembrane catalytic \(\alpha\) subunit has a molecular weight of 100,000 and transports two Ca\(^{2+}\) ions per ATP hydrolyzed. In the cytosol of muscle cells, the free Ca\(^{2+}\) concentration ranges from \(10^{-7}\) M (resting cells) to more than \(10^{-6}\) M (contracting cells), whereas the total Ca\(^{2+}\) concentration in the SR lumen can be as high as \(10^{-2}\) M. Sites on the cytosolic surface of the muscle calcium pump have a very high affinity for Ca\(^{2+}\) (\(K_m = 10^{-7}\) M), allowing the pump to transport Ca\(^{2+}\) efficiently from the cytosol into the SR against the steep concentration gradient.

The concentration of free Ca\(^{2+}\) within the sarcoplasmic reticulum is actually much less than the total concentration of \(10^{-2}\) M. Two soluble proteins in the lumen of SR vesicles bind Ca\(^{2+}\) and serve as a reservoir for intracellular Ca\(^{2+}\), thereby reducing the concentration of free Ca\(^{2+}\) ions in the SR vesicles, and consequently decreasing the energy needed to pump Ca\(^{2+}\) ions into them from the cytosol. The activity of the muscle Ca\(^{2+}\) ATPase is so regulated that if the free Ca\(^{2+}\) concentration in the cytosol becomes too high, the rate of calcium pumping increases until the cytosolic Ca\(^{2+}\) concentration is reduced to less than 1 \(\mu\)M. Thus in muscle cells, the calcium pump in the SR membrane can supplement the activity of the plasma-membrane pump, assuring that the cytosolic concentration of free Ca\(^{2+}\) remains below 1 \(\mu\)M.

The current model of the mechanism of action of the Ca\(^{2+}\) ATPase in the SR membrane is outlined in Figure 15-11. Coupling of ATP hydrolysis with ion pumping involves several steps that must occur in a defined order. When the protein is in one conformation, termed \(E_1\), two Ca\(^{2+}\) ions bind in sequence to high-affinity sites on the cytosolic surface (step 1). Then an ATP binds to its site on the cytosolic surface; in a reaction requiring that a Mg\(^{2+}\) ion be tightly complexed to the ATP, the bound ATP is hydrolyzed to ADP and the liberated phosphate is transferred to a specific aspartate residue in the protein, forming a high-energy acyl phosphate bond, denoted by \(E_1\)–\(P\) (step 2). The protein then changes its conformation to \(E_2\)–\(P\), generating two low-affinity Ca\(^{2+}\)-binding sites on the exoplasmic surface, which faces the SR lumen; this conformational change simultaneously propels the two Ca\(^{2+}\) ions through the protein to these sites (step 3) and inactivates the high-affinity Ca\(^{2+}\)-binding sites on the cytosolic face. The Ca\(^{2+}\) ions then dissociate from the exoplasmic surface of the protein (step 4). Following this, the aspartyl-phosphate bond in \(E_2\)–\(P\) is hydrolyzed, causing \(E_2\) to revert to \(E_1\), a change that inactivates the exoplasmic-facing Ca\(^{2+}\)-binding sites and regenerates the cytosolic-facing Ca\(^{2+}\)-binding sites (step 5).
**Figure 15-11**

Model of the mechanism of action of muscle Ca\(^{2+}\) ATPase, which is located in the sarcoplasmic reticulum (SR) membrane. Only one of the two α subunits of this P-class pump is depicted. E1 and E2 are alternate conformational forms of the protein (more...)

Thus phosphorylation of the muscle calcium pump by ATP favors conversion of E1 to E2, and dephosphorylation favors the conversion of E2 to E1. While only E2 – P, not E1~P, is actually hydrolyzed, the free energy of hydrolysis of the aspartyl-phosphate bond in E1~P is greater than that for E2 – P. The reduction in free energy of the aspartyl-phosphate bond in E2 – P, relative to E1~P, can be said to power the E1 → E2 conformational change. The affinity of Ca\(^{2+}\) for the cytosolic-facing binding sites in E1 is a thousandfold greater than the affinity of Ca\(^{2+}\) for the exoplasmic-facing sites in E2; this difference enables the protein to transport Ca\(^{2+}\) unidirectionally from the cytosol, where it binds tightly to the pump, to the exoplasm, where it is released.

Much evidence supports the model depicted in Figure 15-11. For instance, the muscle calcium pump has been isolated with phosphate linked to an aspartate residue, and spectroscopic studies have detected slight alterations in protein conformation during the E1 → E2 conversion. On the basis of the protein’s amino acid sequence and various biochemical studies, investigators proposed the structural model for the catalytic α subunit shown in Figure 15-12. The membrane-spanning α helices are thought to form the passageway through which Ca\(^{2+}\) ions move. The bulk of the subunit consists of cytosolic globular domains that are involved in ATP binding, phosphorylation of aspartate, and energy transduction. These domains are connected by “stalks” to the membrane-embedded domain.

**Figure 15-12**

Schematic structural model for the catalytic (α) subunit of muscle Ca\(^{2+}\) ATPase. The 10 transmembrane α helices are thought to form a channel through which Ca\(^{2+}\) ions move. Site-specific mutagenesis studies have identified four residues (more...)

As noted previously, all P-class ion pumps, regardless of which ion they transport, are phosphorylated during the transport process. The amino acid sequences around the phosphorylated aspartate in the catalytic α subunit are highly conserved in all proteins of this type. Thus the mechanistic model in Figure 15-11 probably is generally applicable to all these ATP-powered ion pumps. In addition, the α subunits of all the P pumps examined to date have a similar molecular weight and, as deduced from their amino acid sequences derived from cDNA clones, have a similar arrangement of transmembrane α helices (see Figure 15-12). These findings strongly suggest that all these proteins evolved from a common precursor, although they now transport different ions.

Go to:
Na+/K+ ATPase Maintains the Intracellular Na+ and K+ Concentrations in Animal Cells

A second P-class ion pump that has been studied in considerable detail is the Na+/K+ ATPase present in the plasma membrane of all animal cells. This ion pump is a tetramer of subunit composition α₂β₂. (Classic Experiment 15.1 describes the discovery of this enzyme.) The β polypeptide is required for newly synthesized α subunits to fold properly in the endoplasmic reticulum but apparently is not involved directly in ion pumping. The α subunit is a 120,000-MW nonglycosylated polypeptide whose amino acid sequence and predicted membrane structure are very similar to those of the muscle Ca²⁺ ATPase. In particular, the Na+/K+ ATPase has a stalk on the cytosolic face that links domains containing the ATP-binding site and the phosphorylated aspartate to the membrane-embedded domain. The overall process of transport moves three Na⁺ ions out of and two K⁺ ions into the cell per ATP molecule split (Figure 15-13a).

Figure 15-13
Models for the structure and function of the Na⁺/K⁺ ATPase in the plasma membrane. (a) This P-class pump comprises two copies each of a small glycosylated β subunit and a large α subunit, which performs ion transport. Hydrolysis of one (more...)

Several lines of evidence indicate that the Na⁺/K⁺ ATPase is responsible for the coupled movement of K⁺ and Na⁺ into and out of the cell, respectively. For example, the drug ouabain, which binds to a specific region on the exoplasmic surface of the protein and specifically inhibits its ATPase activity, also prevents cells from maintaining their Na⁺/K⁺ balance. Any doubt that the Na⁺/K⁺ ATPase is responsible for ion movement was dispelled by the demonstration that the enzyme, when purified from the membrane and inserted into liposomes, propels K⁺ and Na⁺ transport in the presence of ATP.

The mechanism of action of the Na⁺/K⁺ ATPase, outlined in Figure 15-13b, is similar to that of the muscle calcium pump, except that ions are pumped in both directions across the membrane. In its E1 conformation, the Na⁺/K⁺ ATPase has three high-affinity Na⁺-binding sites and two low-affinity K⁺-binding sites on the cytosolic-facing surface of the protein. The Kₘ for binding of Na⁺ to these cytosolic sites is 0.6 mM, a value considerably lower than the intracellular Na⁺ concentration of ≈12 mM; as a result, Na⁺ ions normally will fill these sites. Conversely, the affinity of the cytosolic K⁺-binding sites is low enough that K⁺ ions, transported inward through the protein, dissociate from E1 into the cytosol despite the high intracellular K⁺ concentration. During the E1 → E2 transition, the three bound Na⁺ ions move outward through the protein. Transition to the E2 conformation also generates two high-affinity K⁺ sites and three low-affinity Na⁺ sites on the exoplasmic face. Because the Kₘ for K⁺ binding to these sites (0.2 mM) is considerably lower than the extracellular K⁺ concentration (4 mM), these sites will fill quickly...
with K⁺ ions. In contrast, the three Na⁺ ions, transported outward through the protein, will dissociate into the extracellular medium from the low-affinity Na⁺ sites on the exoplasmic surface despite the high extracellular Na⁺ concentration. Similarly, during the E2 → E1 transition, the two bound K⁺ ions are transported inward.

V-Class H⁺ ATPases Pump Protons across Lysosomal and Vacuolar Membranes

All V-class ATPases transport H⁺ ions only. These proton pumps, present in the membranes of lysosomes, endosomes, and plant vacuoles, function to acidify the lumen of these organelles. The acidity of the lysosomal lumen, usually ≈4.5 – 5.0, can be measured precisely in living cells by use of particles labeled with a pH-sensitive fluorescent dye. Cells phagocytose these particles (see Figure 5-44a) and transfer them to the lysosomes. The ability of different wavelengths of visible light to excite fluorescence is highly dependent on pH, and the lysosomal pH can be calculated from the spectrum of the fluorescence emitted. Maintenance of the 100-fold or more proton gradient between the lysosomal lumen (pH ≈4.5 – 5.0) and the cytosol (pH ≈7.0) depends on ATP production by the cell.

The ATP-powered proton pumps in lysosomal and vacuolar membranes have been isolated, purified, and incorporated into liposomes. As illustrated in Figure 15-10, these V-class proton pumps contain two discrete domains: a cytosolic-facing hydrophilic domain (V₁) composed of five different polypeptides and a transmembrane domain (V₀) containing 9 – 12 copies of proteolipid c, one copy of protein b, and one copy of protein a. The subunit composition of the cytosolic domain is α₃β₃γδε; the α and β subunits contain the sites where ATP binding and hydrolysis occur. Each transmembrane c subunit is thought to span the membrane two times; the c and a subunits together form the proton-conducting channel. Unlike P-class ion pumps, the V-class H⁺ ATPases are not phosphorylated and dephosphorylated during proton transport.

Similar V-class ATPases are found in the plasma membrane of certain acid-secreting cells. These include osteoclasts, bone-resorbing macrophagelike cells, which bind to a bone and seal off a small segment of extracellular space between the plasma membrane and the surface of the bone. HCl secreted into this space by osteoclasts dissolves the calcium phosphate crystals that give bone its rigidity and strength.

Another example is the mitochondria-rich epithelial cells lining the toad bladder; the apical plasma membrane of these cells contain many V-class H⁺ ATPases, which function to acidify the urine (Figure 15-14). As we discuss later, the membrane of plant vacuoles contains two proton pumps: a typical V-class H⁺ ATPase and another one that utilizes the energy released by hydrolysis of inorganic pyrophosphate (PPᵢ) to pump protons into the vacuole. This PPᵢ-hydrolyzing proton pump, believed to be unique to plants, has an amino acid sequence different from any other ion-transporting proteins.
The plasma membrane of certain acid-secreting cells contains an almost crystalline array of V-class H\(^+\) ATPases. This electron micrograph is of a platinum replica of the cytosolic surface of the apical plasma membrane of a toad bladder epithelial cell. (more...)

ATP-powered proton pumps cannot acidify the lumen of an organelle (or the extracellular space) by themselves. The reason for this is that pumping of protons would rapidly cause a buildup of positive charge on the exoplasmic face of the membrane on the inside of the vesicle membrane and a corresponding buildup of negative charges on the cytosolic face. In other words, the pump would generate a voltage across the membrane, exoplasmic face positive, which would prevent movement of protons into the vesicle before a significant H\(^+\) concentration gradient had been established. In fact, this is the way that H\(^+\) pumps generate an insidenegative potential across plant and yeast plasma membranes. In order for an organelle lumen or an extracellular space (e.g., the outside of an osteoclast) to become acidic, movement of H\(^+\) up its concentration gradient must be accompanied by (1) movement of an equal number of anions in the same direction or (2) movement of equal numbers of a different cation in the opposite direction. The first process occurs in lysosomes and plant vacuoles whose membranes contain V-class H\(^+\) ATPases and ion channels through which accompanying anions (e.g., Cl\(^-\)) move. The second occurs in the lining of the stomach, which contains a P-class H\(^+\)/K\(^+\) ATPase that pumps one H\(^+\) outward and one K\(^+\) inward.

Go to:

The ABC Superfamily Transports a Wide Variety of Substrates

As noted earlier, all members of the very large and diverse ABC superfamily of transport proteins contain two transmembrane (T) domains and two cytosolic ATP-binding (A) domains (see Figure 15-10). The T domains, each built of six membrane-spanning \(\alpha\) helices, form the pathway through which the transported substance (substrate) crosses the membrane and determine the substrate specificity of each ABC protein. The sequence of the A domains is \(\approx 30\) to \(40\) percent homologous in all members of this superfamily, indicating a common evolutionary origin. Some ABC proteins also contain a substrate-binding subunit or regulatory subunit.

Bacterial Plasma-Membrane Permeases

The plasma membrane of many bacteria contain numerous permeases that belong to the ABC superfamily. These proteins use the energy released by hydrolysis of ATP to transport specific amino acids, sugars, vitamins, or even peptides into the cell. Since bacteria frequently grow in soil or pond water where the concentration of nutrients is low, these ABC transport proteins allow the cells to concentrate amino acids and other nutrients in the cell against a substantial concentration gradient. Bacterial permeases generally are inducible; that is, the quantity of a transport protein in the cell membrane is regulated by both the concentration of the nutrient in the medium and the metabolic needs of the cell.

In *E. coli* histidine permease, a typical bacterial ABC protein, the two transmembrane domains and two cytosolic ATP-binding domains are formed by four separate subunits. In gram-negative bacteria such as *E. coli*, which have an outer membrane, a soluble histidine-binding protein in the
periplasmic space assists in transport (Figure 15-15). This soluble protein binds histidine tightly and directs it to the T subunits, through which histidine crosses the membrane powered by ATP hydrolysis. Mutant *E. coli* cells that are defective in any of the histidine-permease subunits or the soluble binding protein are unable to transport histidine into the cell, but are able to transport other amino acids whose uptake is facilitated by other transport proteins. Such genetic analyses provide strong evidence that histidine permease and similar ABC proteins function to transport solutes into the cell.

**Figure 15-15**

Gram-negative bacteria import many solutes by means of ABC proteins (permeases) that utilize a soluble substrate-binding protein present in the periplasmic space. Depicted here is the import of the amino acid histidine. After diffusing through porins (more...)

**Mammalian MDR Transport Proteins**

A series of rather unexpected observations led to discovery of the first eukaryotic ABC protein. Oncologists noted that tumor cells often became simultaneously resistant to several chemotherapeutic drugs with unrelated chemical structures; similarly, cell biologists observed that cultured cells selected for resistance to one toxic substance (e.g., colchicine, a microtubule inhibitor) frequently became resistant to several other drugs, including the anticancer drug adriamycin. Subsequent studies showed that this resistance is due to enhanced expression of a multidrug-resistance (MDR) transport protein known as MDR1. In this member of the ABC superfamily, all four domains are “fused” into a single 170,000-MW protein (Figure 15-16). This protein uses the energy derived from ATP hydrolysis to export a large variety of drugs from the cytosol to the extracellular medium. The *Mdr1* gene is frequently amplified in multidrug-resistant cells, resulting in a large overproduction of the MDR1 protein.

**Figure 15-16**

Schematic structural model for mammalian MDR1 protein. In this member of the ABC superfamily, the two transmembrane domains and two cytosolic ATP-binding domains are part of a single polypeptide. Each transmembrane domain contains six α helices. (more...)

Most drugs transported by MDR1 are small hydrophobic molecules, which diffuse from the culture medium across the plasma membrane into the cell. The ATP-powered export of such
drugs from the cytosol by MDR1 means a much higher extracellular drug concentration is required to kill cells. That MDR1 is an ATP-powered small-molecule pump has been demonstrated with liposomes containing the purified protein (see Figure 15-4). The ATPase activity of these liposomes is enhanced by different drugs in a dose-dependent manner corresponding to their ability to be transported by MDR1.

Not only does MDR1 transport a varied group of molecules, but all these substrates compete with one another for transport by MDR1. Although the mechanism of action of MDR1-assisted transport has not been definitively demonstrated, the flippase model, depicted in Figure 15-17a, is a likely candidate. Substrates of MDR1 are primarily planar, lipid-soluble molecules with one or more positive charges, and they move spontaneously from the cytosol into the cytosolic-facing leaflet of the plasma membrane. The hydrophobic portion of a substrate molecule is oriented toward the hydrophobic core of the membrane, and the charged portion toward the polar cytosolic face of the membrane and is still in the cytosol. The substrate diffuses laterally until encountering and binding to a site on the MDR1 protein that is within the bilayer. The protein then “flips” the charged substrate molecule into the exoplasmic leaflet, an energetically unfavorable reaction powered by the coupled ATPase activity of MDR1. Once in the exoplasmic face, the substrate diffuses into the aqueous phase on the outside of the cell.

Support for the flippase model of transport by MDR1 comes from MDR2, a homologous protein present in the region of the liver cell plasma membrane that faces the bile duct. MDR2 has been shown to flip phospholipids from the cytosolic-facing leaflet of the plasma membrane to the exoplasmic leaflet, thereby generating an excess of phospholipids in the exoplasmic leaflet; these phospholipids peel off into the bile duct and form an essential part of the bile. An alternative pump model also has been proposed for MDR1 (Figure 15-17b). According to this model, drug molecules in the cytosol bind directly to a single small-molecule binding site on the cytosolic face of the MDR1 protein; subsequent ATP hydrolysis powers movement of the bound drug through the protein to the aqueous phase on the outside of the cell by a mechanism similar to that of other ATP-powered pumps.

**Figure 15-17**

Possible mechanisms of action of the MDR1 protein. (a) The flippase model proposes that a lipid-soluble molecule first dissolves in the cytosolic-facing leaflet of the plasma membrane and then diffuses in the membrane until binding (more...)

MDR1 protein is expressed in abundance in the liver, intestines, and kidney — sites from which natural toxic products are removed from the body. Thus the natural function of MDR1 may be to transport a variety of natural and metabolic toxins into the bile, intestinal lumen, or forming urine. During the course of its evolution, MDR1 appears to have coincidentally acquired the ability to transport drugs whose structures are similar to those of these toxins. Tumors derived from these cell types, such as hepatomas (liver cancers), frequently are resistant to virtually all chemotherapeutic agents and thus difficult to treat, presumably because the tumors exhibit increased expression of the MDR1 or MDR2 proteins.
Cystic Fibrosis Transmembrane Regulator (CFTR) Protein

Discovery of another ABC transport protein came from studies of cystic fibrosis (CF), the most common lethal autosomal recessive genetic disease of Caucasians. This disease is caused by a mutation in the CFTR gene, which encodes a chloride-channel protein that is regulated by cyclic AMP (cAMP), an intracellular second messenger. These Cl– channels are present in the apical plasma membranes of epithelial cells in the lung, sweat glands, pancreas, and other tissues. An increase in cAMP stimulates Cl– transport by such cells from normal individuals, but not from CF individuals who have a defective CFTR protein.

The sequence and predicted structure of the encoded CFTR protein, based on analysis of the cloned gene, are very similar to those of MDR1 protein except for the presence of an additional domain, the regulatory (R) domain, on the cytosolic face. The Cl– channel activity of CFTR protein clearly is enhanced by binding of ATP. Moreover, as detailed in Chapter 20, cAMP activates a protein kinase that phosphorylates, and thereby activates, CFTR. When purified CFTR protein is incorporated into liposomes, it forms Cl– channels with properties similar to those in normal epithelial cells. And when the wild-type CFTR protein is expressed by recombinant techniques in cultured epithelial cells from CF patients, the cells recover normal Cl– channel activity. This latter result raises the possibility that gene therapy might reverse the course of cystic fibrosis.

Since CFTR protein is similar to MDR1 in structure, it may also function as an ATP-powered pump of some as-yet unidentified molecule. In any case, much remains to be learned about this fascinating class of ABC transport proteins.

Regulation and Coordination of Intracellular Trafficking: An Overview

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Trafficking Inside Cells: Pathways, Mechanisms and Regulation, edited by Nava Segev, Editor, with Associate Editors: Aixa Alfonso, Gregory Payne and Julie Donaldson.


Read this chapter in the Madame Curie Bioscience Database here.
During the last two decades, efforts in the protein trafficking field have focused primarily on the identification of the machinery components of vesicular transport and mechanisms that underlie it. In addition, research has started to reveal how intracellular trafficking is regulated. Here, we summarize the current state of our knowledge about the regulation of vesicular transport and its coordination with other cellular processes. At the most basic level, individual transport steps are regulated spatially and temporally in two different ways. First, molecular switches of the Arf, Rab and Rho GTPase families regulate the assembly of components of the vesicular transport machinery on membranes, mediating the formation, targeting and fusion of vesicles that shuttle cargo between intracellular compartments. Second, reversible posttranslational modifications, like phosphorylation and ubiquitination, allow efficient cargo sorting and machinery component recycling. At a higher level, individual transport steps are integrated into whole pathways, with GTPases as a mechanism for this integration. Finally, intracellular trafficking pathways are coordinated with other cellular processes. Here too, GTPases appear to play a role by orchestrating coordination.

Go to:

**Introduction**

Eukaryotic cells have a complex array of exocytic and endocytic membrane systems. Movement of membranes and cargos between organelles must occur efficiently while maintaining the integrity and structure of the organelles. Such maintenance requires sorting of proteins for forward transport while retaining resident proteins, as well as recycling of membranes and resident proteins back to donor organelles. Our current knowledge of the intracellular compartments and pathways are the subject of the first section of this book.\(^1\) Transport between organelles is mediated by membrane-bounded vesicles, which move membranes and proteins in both directions. Identification of the machinery components of vesicular transport and the mechanisms by which they function, the major issue the field has dealt with during the last two decades, is summarized in Section II. The progress made in these studies has made it possible to embark on the next major challenge in this field: understanding the spatial and temporal regulation of vesicular transport and the integration of individual transport steps into whole pathways in the context of the cell. This topic is the subject of Section III.

Two different mechanisms regulate individual vesicular transport steps. The first involves monomeric GTPases that act as molecular switches. These proteins regulate all aspects of vesicle life, from formation at the donor compartment to fusion with the acceptor compartment and are the subject of Chapter 16.\(^2\) The second type of regulatory mechanism uses posttranslational modifications, i.e., phosphorylation and ubiquitination of proteins. The best-characterized examples of this type of regulation occur in the endocytic pathway, where both endocytic cargo and endocytic machinery components are modified in a reversible way to allow cargo sorting and machinery component recycling. This type of regulation is discussed in Chapter 17.\(^3\)

Individual transport steps require coordination to allow integration of the steps into whole pathways. Monomeric GTPases and their upstream regulators play a key role in this process too. GTPase cascades were shown to regulate other cellular processes,\(^4\) and there is growing evidence that such cascades act in intracellular trafficking as well.\(^5\)
It has become clear that intracellular trafficking needs to be coordinated with other processes to allow for proper cell function. Evidence of such coordination is beginning to emerge and these examples are discussed in Chapters 18-20. First, intracellular trafficking is important for polarized cell growth. Second, intracellular trafficking is crucial for proper signaling, with Rab GTPases playing a role in this coordination too. Finally, both exocytosis and endocytosis are required for development of multi-cellular organisms.

Here, we summarize what is currently known about the regulation of intracellular trafficking, its coordination with other processes and the importance of this regulation to human health, and we discuss future perspectives in this field.

Go to:

**Regulation of Individual Transport Steps**

Components of the trafficking machinery cannot by themselves drive efficient vesicular transport. For example, specific SNARE combinations can drive synthetic membrane fusion; however, the fusion reaction is extremely slow. Two types of highly conserved regulations ensure that intracellular trafficking is a specific and efficient process: monomeric GTPases and posttranslational modifications of cargo and machinery components.

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**GTPases Regulating Individual Vesicular Transport Steps**

Monomeric GTPases of the Arf, Rab, Rho and dynamin families control specific vesicular transport steps. GTPases in general act as molecular switches as they cycle between the inactive GDP-bound and the active GTP-bound forms. This switching is catalyzed by guanine-nucleotide exchange factors (GEFs) that activate the GTPases and by GTPase activating proteins (GAPs) that inactivate them. When in the active state, GTPases that regulate intracellular trafficking interact with downstream effectors. These effectors and their binding proteins mediate the various steps of vesicle life, from formation at the donor compartment to fusion with the acceptor compartment.

Vesicle formation involves a number of processes: coat assembly, cargo sorting, membrane curvature, vesicle fission and vesicle un-coating. All these processes are regulated by GTPases (Fig. 1). Members of the Arf and Rho families regulate assembly of specific coats and coat adaptors required for cargo sorting. For example, Sar1, a member of the Arf family, recruits the ER coat COPII, Arf1 recruits the Golgi coat COPI, and Arf6 and Rho GTPases recruit the clathrin coat at the plasma membrane. Rabs were also suggested to function in vesicle formation and cargo sorting, even though specific coats have not been implicated yet in Rab-mediated vesicle formation. Furthermore, protein coats induce membrane curvature into spherical buds. Therefore, the regulation of coat assembly and disassembly by GTPases plays a role in both cargo sorting and membrane curvature of budding vesicles. Vesicle fission at the neck is mediated by dynamin GTPases. Finally, Arfs and Rabs were implicated in vesicle un-coating in the exocytic and endocytic pathways, respectively.
Figure 1

Regulation of individual vesicular transport steps by monomeric GTPases from the Arf, Rab, Rho and dynamin families (top).

Members of the Ypt/Rab GTPase family regulate all the steps that follow vesicle formation (Fig. 1). Because individual Ypt/Rabs can recruit multiple effectors, these GTPases can control processes as diverse as vesicle motility, tethering and fusion. Currently, Rabs are envisioned as organizers of membrane micro-domains, definers of compartment identity, and drivers of compartment maturation. These roles might explain the high number of Rabs (70 human Rabs) relative to Arfs (six human Arfs), which are involved only in vesicle formation. Indeed, a global genomic study suggests that Rabs define membrane identity, membrome, of different cell and organ systems.

Progress has been made in recent years in the identification of upstream regulators of GTPases that control intracellular trafficking. For the Arf and Rho families, numerous GEFs and GAPs have been identified. All Arf GEFs contain a Sec7 domain, whereas Rho GEFs contain the Dbl homology (DH) domain; these domains comprise the catalytic core of the GEFs. There are numerous Arf GAPs that can be identified by a Zn-finger GAP domain in addition to other regulatory and protein-protein interaction domains. Similarly, a conserved Ypt/Rab GAP domain allows the identification of multiple Rab GAPs. In contrast, there is a paucity of identified Rab GEFs. The reason for this shortage is that the known Rab GEFs do not share similarity, which makes it harder to identify them. Interestingly, the number of GAPs for GTPases that regulate intracellular trafficking is higher than the number of GEFs. For example, many more mammalian GAPs for Arfs have been identified than Arfs themselves. This observation suggests that either GAPs are more cell- or stage-specific than the GTPases themselves, or that GAPs are also effectors that act in feedback inhibition of their GTPase recruiters.

The major open questions in the GTPase field concern the nature of the molecular mechanisms by which GTPases are regulated and how the GTPases control their downstream effectors. To this end, the full inventory of players is being identified in proteomic studies and molecular mechanisms are being determined in vitro using biochemistry and structural studies, as well as in vivo using knockdown experiments and expression of dominant negative mutations.

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Posttranslational Modifications Regulating Cargo Sorting

Posttranslational modifications (PTMs) regulate sorting of membrane proteins en route to their degradation in lysosomes. This process is important for down regulation of plasma-membrane (PM) receptors and for quality control of membrane proteins in the Golgi and the PM. Two types of PTMs are known to regulate sorting of membrane proteins to endosomes: phosphorylation and
ubiquitination.\textsuperscript{3} Signals for these PTMs are found in the cytoplasmic tails of the membrane proteins and on components of the PTM machinery.

Phosphorylation is required for internalization of a number of PM receptors, notably G-protein coupled receptors (GPCRs). In this case, protein kinases phosphorylate the cytoplasmic tails of receptors, resulting in a signal for interaction with arrestins. Arrestins, in turn, recruit the endocytic machinery, resulting in the internalization and down regulation of the receptors.\textsuperscript{24}

Ubiquitin (Ub) is a highly conserved 76-amino acid polypeptide that can be attached covalently to lysines in other proteins and in Ub itself. Because Ub can be linked to itself, poly-ubiquitin chains can accumulate on cellular proteins. The ubiquitination reaction is carried by a set of enzymes that act successively, with the E3 ubiquitin ligase acting at the end of the reaction. The original role assigned to ubiquitination was for protein degradation in the cytoplasm by proteasomes. However, subsequently ubiquitin was shown to serve as a signal for sorting proteins into endosomes. On endosomes, the ESCRT (endosomal sorting complex required for transport) machinery assembles to target ubiquitinated proteins to intra-luminal vesicles (ILVs) that bud into endosomes, forming multivesicular bodies (MVBs). Fusion of MVBs with lysosomes results in the degradation of the sequestered proteins.

One question that the field addressed is how the Ub signal that sends proteins for degradation in lysosomes is different from the signal that sends proteins to proteasomes. One suggestion was that the difference lies in the numbers of Ub ligands attached to the protein, mono-Ub for endocytosis en route to the lysosome and poly-Ub for sending proteins to the proteasome. Current thinking is that the difference lies not in the number, but in the type of poly-ubiquitination: Ub K48 for the proteosome and Ub K63 for sorting into MVBs. Endocytosis-related ubiquitination is performed by the conserved Rsp5/Nedd4 Ub ligase. This ligase recognizes PY motifs on the cytoplasmic tails of membrane proteins, or on adaptors that attach to these tails in the Golgi and the PM.

Both phosphorylation and ubiquitination are reversible PTMs. This reversibility might be required for cargo sorting to lysosomes, i.e., the Ub required for sending proteins to the lysosome has to be removed before the proteins enter this compartment. Alternatively, the reverse reactions might be important because machinery components that perform these PTMs are also modified. For example, the AP-2 clathrin adaptors can be phosphorylated and the Ub-adaptors arrestins, as well as ESCRT subunits, can be ubiquitinated. In this scenario, reversible PTMs are important for the activity of the machinery components or for their recycling.

There are a number of open questions in this field. For example, it is not clear whether phosphorylation and ubiquitination are linked. A recent study suggests that arrestin-related proteins serve as ubiquitin ligase adaptors for PM proteins in yeast.\textsuperscript{25} Because arrestins can recognize the phosphorylated cytoplasmic tails of PM proteins, this finding suggests a link between the two PTMs, phosphorylation and ubiquitination. Other open questions are how ESCRT promotes the formation of ILVs and whether PTMs can sequester proteins into routes other than degradation, e.g., recycling to the PM.\textsuperscript{26}

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Transport Step Coordination

Coordination of individual transport steps can occur at two levels. First, each transport step between any two compartments, e.g., ER-to-Golgi or Golgi-to-PM, involves a number of vesicular transport steps: from vesicle packaging and formation, through its delivery and docking, to its final fusion with the acceptor compartment. These individual vesicular transport steps have to be coordinated. The second level involves integration of individual transport steps of the same pathway, e.g., the various steps of the exocytic and endocytic pathways. Evidence is emerging that monomeric GTPases play a role not only in the regulation of individual vesicular transport steps, but also in the step-integration process.

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Coordination of Individual Vesicular Transport Steps

It makes sense that mechanisms exist for ensuring that a cargo-loaded vesicle that forms at any donor compartment has the capability to be targeted efficiently to the right acceptor compartment and fuse with it. Because monomeric GTPases regulate the multiple individual steps of vesicular transport, they are also obvious candidates for the integration process. The GTPase-dependent cooperation idea was first suggested for the integration of exocytic pathway steps based on genetic studies in yeast. Genetic interactions between Arf GEFs and Ypt/Rabs suggested coupling between Arf-dependent vesicle formation with Ypt/Rab-dependent vesicle targeting and fusion.27

More recently, a number of specific GTPase cascades that couple vesicular transport steps were described (Fig. 2). In the exocytic pathway, interaction of the Golgi coiled-coil protein p115 with the Arf GEF GBF1, Rab1 and SNAREs was suggested as a way for integrating ER-to-Golgi vesicle formation, tethering and fusion in mammalian cells.28 Another example of cooperation between two Ypt/Rab GTPases that regulate individual vesicular transport steps of Golgi-to-PM transport has also been shown in yeast. In this case, the Ypt31/32 functional pair required for Golgi vesicle formation and motility13,29 interacts with Sec2, a GEF for the Ypt/ Rab Sec4, which is required for the fusion of these vesicles with the PM.30

Figure 2

Coordination of individual vesicular transport steps by GTPases and their accessory factors: (A) The mammalian Golgin p115 interacts with GBF1, a GEF for Arf GTPase, as well as Rab1 GTPase, and SNAREs, to coordinate formation, tethering and fusion of (more...) Two recent papers suggest cooperation between GTPases in both packaging and tethering of endosome-to-TGN vesicles. The first paper demonstrates that cooperation between Rab5 and Rab7 is required for recruitment of the two parts of the retomer complex. These two parts of the retromer are needed for formation of vesicles containing the mannose 6-phosphate receptor
A second paper identifies interaction of the golgin GCC185 with multiple GTPases, Rab9, Rab6 and Arl1, as a requirement for the tethering of MPR-containing vesicles to the TGN.

Integration of Individual Transport Steps into Whole Pathways

To ensure unobstructed transport flow through a pathway as well as maintenance of compartment size, individual steps must be coordinated. Evidence of such coordination by monomeric GTPases and their GEF activators is beginning to emerge. One example is sequential activation of Ypt/Rab GTPases that regulate Golgi entry and exit in yeast by the modular GEF/tethering complex TRAPP. TRAPP is found on the Golgi in two confirmations: TRAPPI at the cis Golgi and TRAPPII at the trans Golgi. The finding that these two complexes act as GEFs for the Golgi Ypt/Rab gatekeepers, Ypt1 and Ypt31/32, raises the exciting possibility that sequential activation of the Ypt/Rabs coordinates Golgi entry and exit. Another example of a Rab cascade was suggested to drive endosome maturation. In this case, a conversion of early-to-late endosome is driven by Rab5 on early endosomes, recruiting the GEF for the late-endosome organizer Rab7.

Coordination of Intracellular Trafficking with Other Cellular Processes

Proper cell function requires the coordination of all cellular processes, including intracellular traffic. This section describes the mechanisms by which intracellular trafficking is coordinated with cell polarization, efficient signal transduction and development.

In polarized cells, compartments and functions are distributed asymmetrically. Therefore, during the establishment of cell polarity, PM symmetry has to be broken and the newly established asymmetry has to be maintained. In yeast, cell polarity is important for both asymmetric cell division, and for response to mating pheromones. In multi-cellular organisms, cell polarization is important for the functioning of polarized tissues; e.g., asymmetry of the apical and basolateral surfaces is required for epithelial cell function, asymmetry of the axonal and dendritic sides is important for neuronal cell function, and asymmetry during stem cell division is crucial for their differentiation. Consequently, disturbance of cell polarity can result in cancer, problems in transmission of information in the brain and developmental abnormalities.

Establishment of cell polarity requires coordination between a number of cellular machineries (Fig. 3). First, polarity cues must be positioned on the PM in response to internal or external signals. Second, the polarity cues have to be decoded and the actin cytoskeleton and the exocytic pathway have to reorient towards these positional cues. Third, maintenance of cell polarity depends on microtubules. Exocytosis and endocytosis are required for breaking the cell symmetry and for maintaining the asymmetry.
Figure 3
Coupling of intracellular trafficking with the establishment of cell polarity. Monomeric GTPases from the Rap, Rho, Ral and Rab families play key roles in all steps.

Monomeric GTPases play a role in the establishment of cell polarity and its coordination with intracellular trafficking (Fig. 3). These GTPases regulate the positioning of the cues, the reorientation of the actin cytoskeleton and the coordination between the actin cytoskeleton and the trafficking machinery. In budding yeast, a GTPase cascade plays a role in this coordination. The Rap GTPase Bud1 and its upstream regulators play a role in positioning cues for the emerging bud. Bud1 recruits another GTPase, Cdc42. The Rho GTPase Cdc42 and its downstream effectors are required for establishing a landmark for the actin cytoskeleton reorientation and polarized secretion. In the latter process, the exocyst complex, which serves as a tether between secretory vesicles and the PM, plays a role in the special regulation of exocytosis. Rab, Ral and Rho GTPases regulate the assembly and activation of the exocyst. All these components are conserved in all eukaryotes and the emerging basic mechanisms are similar in all eukaryotic cells.

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Intracellular Trafficking and Signal Transduction

Transduction of external signals through the PM is essential for the interaction of cells with their environment. In this process, receptors present on the cell surface bind external ligands, such as growth factors, neurotransmitters or hormones, and transduce the signal to the inside of the cell. This process is crucial for the functioning of all cells, tissues and organs, and its disruption results in aberrant cell growth and function leading to human disease. Interdependence between intracellular trafficking and signaling is key for proper response to external signals (Fig. 4).

Figure 4
Interdependence between signaling and intracellular trafficking. Specific Rab GTPases control each process: Signaling stimulates Rab5-mediated endocytosis, and activated receptors can continue to signal on endosomes. The balance between Rab4/11-mediated (more...)

On the one hand, signal transduction also regulates endocytosis (Fig. 4). For example, a stimulated G-protein coupled receptor (GPCR) interacts with β-arrestin. This interaction induces the assembly of the endocytic machinery and internalization of the GPRC receptor. Activation of
endocytosis by signaling is achieved through regulation of endocytic Rabs. For example, activation of the EGFR leads to the activation of Rab5 and stimulation of endocytosis, thus leading to internalization of the EGF receptor.³

Conversely, the endocytic pathway is required for signaling regulation. The established role for endocytosis in signaling is in the internalization of ligand-bound receptors, which serves as a mechanism for signaling down-regulation. Internalized receptors are delivered to early endosomes and then can be either recycled back to the PM for further signaling or transported to lysosomes for degradation. The balance between receptor recycling and degradation determines signaling amplitude and duration. Therefore, Rab GTPases that regulate endocytosis play an important role in the regulation of signal transduction (Fig. 4).

In addition, there is evidence that signaling events occur not only on the PM, but also on endosomes. For example, the internalized epidermal growth factor receptor (EGFR) remains active and associates with its downstream signaling molecules, like Shc, GRb2 and Sos, on endosomes.³ Moreover, some signaling events require endocytosis. For example, inhibition of endocytosis results in inhibition of some signaling pathways, like the PI3K and ERK1/2 pathways downstream of insulin receptors, but not others, like the insulin receptor Akt pathway.⁴ The functional importance of endocytosis for signaling was recently shown for the stimulation of cell migration by receptor tyrosine kinases (RTKs). Activation of RTKs results in a Rab5-dependent endocytosis of Rac GTPase to endosomes, where Rac is activated by its GEF Tiam1.⁵ Together, these findings imply that in addition to down-regulation, GTPase-dependent endocytosis plays a positive role in signaling (Fig. 4).

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**Intracellular Trafficking and Development**

Development of multi-cellular organisms is regulated at the transcriptional level. However, cell-fate transcriptional regulation depends on the dynamic secretion of signals by some cells and on correct responses to these signals by receiving cells. Therefore, proper regulation of both exocytosis and endocytosis is as important for development as it is for any other process that depends on signaling (see above). In addition, development of some tissues, like epithelia, neurons, and stem cells, requires polarization that also depends on intracellular trafficking (see above). Therefore, a field that interfaces between development and intracellular trafficking has now blossomed. Of particular importance, because development engages multiple tissues and organs, this field involves an extra level of complexity; this complexity is discussed here.

The role of the endocytic pathway in development is well established. During cell-fate decision, gradients of signaling molecules, morphogens, are formed, and the slope of these gradients determine the signaling range. These gradients and their slope depend not only on diffusion, but also on vesicular trafficking of morphogens through cells. For example, formation of a gradient of the *Drosophila* TGF-β homologue Dpp depends on receptor-mediated endocytosis of this ligand. The slope of the Dpp gradient depends on the ratio between its sorting in endosomes for recycling to the PM or degradation in lysosomes.⁶ Therefore, endocytic Rabs that regulate this ratio play a key role in this process.

A role for exocytosis in development, including cellularization, establishment of polarized tissues and cell-fate determination, is beginning to emerge. During cellularization, the fertilized
egg undergoes synchronous divisions to generate the primary epithilia. Exocytic compartments, like ER and Golgi, and vesicular transport components, like SNAREs, are required for this process. Establishment of polarized tissues, like epithilia and neurons, requires components of the secretory vesicle fusion machinery; e.g., the exocyst complex and PM SNAREs. Cell-fate determination depends on secretion of morphogens from source cells, as well as presentation of receptors on the PM of receiving cells. Both processes depend on exocytosis. For example, in *Drosophila*, the Wnt and Hedgedog morphogens are glycoproteins that also undergo acylation by the acyltransferases Procupine and Ski, respectively. This lipid modification occurs in the ER and is required for the secretion of these morphogens. In addition, a specific chaperone, Evi, is required for shuttling Wnt from the Golgi to the PM.9

Therefore, it is not surprising that there are multiple examples of mutations in genes encoding intracellular components and regulators that result in impaired development. A temperature-sensitive mutation in the *Drosophila* dynamin, shibire, allowed studying the effects of inhibition of endocytosis on development.10 Mutations in the exocytic machinery, like cargo receptors, vesicle coats, tethering factors and SNAREs, result in developmental defects in *Drosophila*, mice and humans. Finally, mutations in GTPases from the Arf/Sar1 and Rab families that regulate the endocytic and exocytic pathways also affect development.2

**Traffic Regulation and Human Disease**

Impairment of secretion of substances like hormones, antibodies and neuro-transmitters, defects in presentation of receptors on the plasma membrane, and obstruction of uptake of ligands from the environment can result in malfunctioning of various body systems and, therefore, can cause human diseases. Thus, it is expected that disruption of traffic regulation and coordination would result in human diseases as well. Because down-regulation of PM receptors and quality control of PM transporters and channels are important for the response of cells to environmental signals, the regulation of these processes by PTM has also been implicated in human disease. For example, dysfunction of the ESCRT machinery, which is required for targeting ubiquinated proteins into MVBs, was shown to contribute to cancer and neuro-degeneration.45

In the past few years, malfunctioning of trafficking GTPases and their upstream regulators were implicated in various human disorders. Because GTPases are expressed ubiquitously, it is reasonable that they would be involved in common multifactorial disorders. Indeed, Rabs, Arfs, Rhos and their associated proteins have been implicated in endocrinological diseases like diabetes,46 immunity disorders,42 cancer,42 heart disease,42 and brain disorders like Parkinson’s.42 In addition, GTPases and their associated proteins were also implicated in rare monogenic diseases. This implication is probably due to differential expression of these regulators in specific tissues at specific times.23 Examples include: the ALS2 mutation in a Rab5 GEF is associated with the neurodegenerative disease ALS (Amyotrophic Lateral Sclerosis);51 mutations in Rab27 result in the rare Griscelli syndrome;52 Rab8 was implicated in Huntington disease;53 mutations in Rab25 were linked to cancer aggressiveness.54

Finally, infectious viruses, bacteria and other pathogens can take over the cell by altering the regulation of cellular trafficking for their purposes. Enveloped viruses, like HIV, exploit the ESCRT machinery for their budding.55 Other intracellular pathogens exploit GTPases or their
regulators for their reproduction. Examples include: *Legionella pneumophila* recruit Arf during an early step of its pathogenesis using its own Arf GEF RalF;\(^5^6\) it also expresses its own Rab1 GEF, DrrA, the GAP LepB,\(^5^7\) and the GDF SidM,\(^5^8\) to recruit Rab1 to its membrane. TBC1D20 expressed by the Hepatitis C virus is a Rab1 GAP.\(^5^9\) The obligate pathogens *Chlamydiae* recruit key Rabs into their replication inclusion.\(^6^0\) *Salmonella* expresses SopE to recruit Rab5 to phagosomes as an evasion mechanism of transport into lysosomes.\(^6^1\) The HIV-1 gene HRB, required for viral replication, contains an ARF GAP domain.\(^6^2\) Another HIV-1 gene, Nef, induces Arf6-mediated endocytosis required for MHC-I down-regulation and viral immuno-evasion.\(^6^3\)

In summary, better understanding of how unobstructed intracellular trafficking flow is achieved will directly impinge on our ability to treat human diseases caused by obstruction of this flow. We expect that in the near future GTPases and their associated proteins, as well as trafficking-specific PTM machinery, will emerge as drug intervention targets for both common and rare human diseases and pathogen infections.