# 1 Cell Physiology

Many cells in the body have highly sophisticated electrical signaling mechanisms. These mechanisms allow electrical impulses to travel within and between the cells of the nervous system. Electrical signaling translates ‘thought’ into action by triggering contractions in muscle and facilitates the control of organs within the body by triggering the release of hormones from various endocrine organs.

The proteins that underlie these specialized electrical signaling functions are evolutionarily ancient. In most cases considerable similarity can be found between proteins used by the human brain for electrical signaling and proteins found in single cell prokaryotes. This is because cells, of all kinds, have to solve a common set of basic cell physiological problems. These problems include maintenance of salt balance, maintenance of osmotic balance, transport of useful solutes into the cell and transport of waste products out of the cell. The proteins that underlie these basic cellular functions have been elaborated and modified during the course of evolution to support other complex functions, including the electrical excitability of neurons and muscle cells. This elaboration of protein function by evolution has limits, however, and the molecular complexity of a neuron in a fruit fly brain and one in a human brain is similar. The much larger repertoire of behaviors seen in humans compared to flies is primarily a function of a more complex nervous system rather than the product of significantly more complex molecular and cellular physiology. Neural development in humans has accreted enormous complexity during the course of evolution, largely due to the evolution and elaboration of gene regulatory function.

## Evolution of Cellular Life

The events leading to the evolution of cellular life are very poorly understood. This ancient, apparently unique, historical event cannot be readily replicated, making it inaccessible for systematic study. Consequently, most writing on this topic remains highly speculative, if not hopelessly deluded. Even a fundamental question such as whether the pathway towards life began first with replicating nucleic acids or from a protein based origin currently remains unresolved.

The evolution of the first protocells, small cells with a continuous cell membrane, was a major step in the evolution of life. Before the existence of membrane delimited structures it would have been difficult, or impossible, to define discrete individuals, whatever their biochemical basis. The cell membrane creates the basic delineation between self and non-self, creating the potential for competition between individuals. From the time when individual protocells appeared it is reasonable to believe that the theory of evolution can explain all the subsequent pathways to more complex and diverse life forms. Evolution of the cell membrane represents the dividing line between life, as it is commonly understood, and non-living biochemical processes.

The cell membrane’s critical role in the evolution of single cells comes about because it performs functions that are somewhat analogous to the property and patent laws of a capitalist economy. Like the property laws, the cell membrane distinguishes private property, what is inside the cell, from common property, everything outside the cell. This allows the cell to concentrate useful resources inside the cell (e.g. ATP, glucose) restricting them for the private use of the cell.

An even more important property is that the cell membrane allows the cell to effectively patent any novel innovations occurring in that cell’s genetic material by restricting the sharing of novel proteins and metabolic products. If a cell has an advantageous mutation in its genetic material, that cell and its progeny will retain sole rights to the benefits afforded by that mutation for some time, potentially conferring a competitive advantage to cells in this lineage relative to other cells. The cell membrane can function in this way because it is an effective barrier to the transport of charged and polar molecules.

### Physical Constraints on Cellular Physiology

Although the steps leading to the evolution of membrane bound, cell based life are not well understood there are several well defined physical constraints that definitely had to be resolved before this could occur. Three particular problems were:

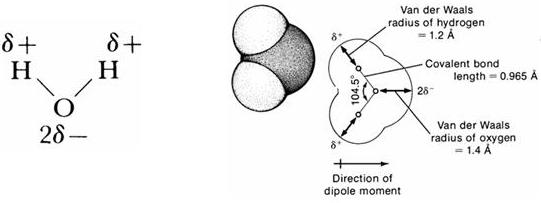
1. Transport of metabolites across the cell membrane
2. Regulation of internal calcium concentration
3. Regulation of osmotic balance/cell volume

The solutions selected for these problems set the basic plan for all subsequent cellular life and reverberate to the present day. Much of the particular functionality of the neurons in your brain derive from the solutions selected several billion years ago to resolve these narrow apparently simple physical problems. The rest of this chapter and the next describe the basic solutions to these problems.

## Water

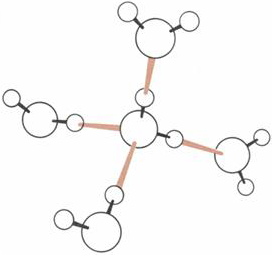
It is difficult to ignore the central role of water in virtually every aspect of cell physiology. Water is essential for life, as we understand the term. The human body is composed primarily of water molecules, which make up about 60% of the total body weight and 99% of the total number of molecules.

Water is a polar molecule, meaning that there is an uneven distribution of charge within the water molecule. The bonds between the oxygen and two hydrogen atoms are polar covalent bonds. The polarity of the covalent bonds results from the high **electronegativity** of the oxygen atom relative to the hydrogen atom. As a consequence, a partial charge distribution exists such that there is a local positive charge on each hydrogen atom and a partial negative charge on the oxygen atom. The uneven distribution of electrons due to the nature of the H-O bond causes the water molecule to act as a **dipole**, meaning that the molecule has a positive and negative pole (Figure **1**).



##### **Figure 1** Dipole nature of water, a polar molecule. There is a partial negative charge (2δ-) on the oxygen atom and partial positive charges (δ+) on the two hydrogen atoms.

Water can form hydrogen bonds between the positively charged hydrogen atoms and negatively charged oxygen atoms in the neighboring water molecules. Since the angle between the two covalent bonds of water is about 105°, groups of hydrogen-bonded water molecules form tetrahedral arrangements (Figure **2**).



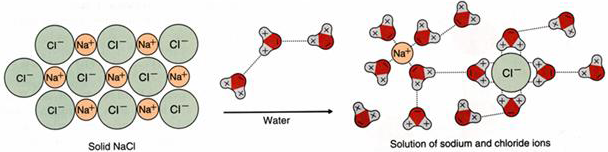
##### **Figure 2** Hydrogen bonding between water molecules.

By itself pure water is not a good conductor of electricity. The conduction of electrical current by an aqueous solution depends primarily on the number and nature of the charged ions found in that solution. These ions can dissolve in water because of the polar nature of water molecules. Water is generally a good **solvent** for ionic compounds, which include salts, acids and bases. These solutes all share the property of **dissociating** into ions when dissolved in water.

## Ions

### Ions in Solution

Two of the most common ions in the body are Na+ and Cl-. NaCl is common table salt. The need to replenish these ions in the body is one reason why salty foods are perceived by animals as tasty. The NaCl molecule is uncharged and cannot carry a current in its undissociated form. In the NaCl crystal, the Na+ and Cl- ions are firmly held together by ionic bonds between the positively charged Na+ ions and the negatively charged Cl- ions (Figure **3**).



##### **Figure 3** Dissociation of Na+ and Cl- ions in water.

Water can dissolve the NaCl crystal, just as it can dissolve most other ionic compounds (salts, acids, bases), because the dipolar water molecules can overcome the electrostatic interactions between the individual ions. The partial negative charge of the oxygen allows weak electrostatic binding with the positively charged Na+ ion and the partial positive charge on the hydrogen allows weak electrostatic binding with the negatively charged Cl- ion. The water molecules surround the ions, orienting themselves so that their positive poles face anions and their negative poles face cations. These shells of water molecules are known as **hydration shells** (Figure 3).

Dissociated ions are the primary current carriers in the body. They are known collectively as **electrolytes**, because of their ability to conduct electricity. Ions that have a net positive charge are called **cations**, while those that have a net negative charge are called **anions**. A failure of athletes to replenish electrolytes during endurance or other sporting events can produce disruption of the electrical activity in some of their electrically excitable cells, with potentially tragic effect if cardiac function is disrupted.

### Distribution of Ions in the Body

The water in the body can be divided into two main compartments: intracellular and extracellular. These two compartments are separated by the cell membranes of individual cells. Typical values for the concentration of the most common ions in these two compartments are given in Table 1. These specific values will vary among different species and between different cells in the body but the general principle is that all animal cells have a relatively high intracellular concentration of K+ ions, a low intracellular concentration of Na+ ions and a very low intracellular concentration of Ca2+ ions. The high concentration of NaCl in the extracellular fluid is similar to sea water and reflects our origins as ocean living organisms. There is a relatively high concentration of fixed anions inside the cell. These comprise all the organic compounds synthesized or sequestered by the cell, which have a net negative charge.

|  |  |  |
| --- | --- | --- |
| Ion | Intracellular (mM) | Extracellular (mM) |
| K+ | 125 | 5 |
| Na+ | 12 | 120 |
| Cl- | 5 | 125 |
| Ca2+ | 1 x10-4 (100 nM) | 2 |
| A- | 108 | 0 |

##### **Table 1.** Ion concentrations in the intracellular and extracellular fluids of a typical mammalian cell. A- = the fixed anions, sum of all the proteins, amino acids (aspartate and glutamate), inorganic ions (sulfate and phosphate), nucleotides, DNA, RNA that are located inside the cell.

Maintenance of this unequal distribution of ions between the inside and outside of the cell is a primary function of the cell and dictates much of the basic cellular physiology of every cell.

## Diffusion

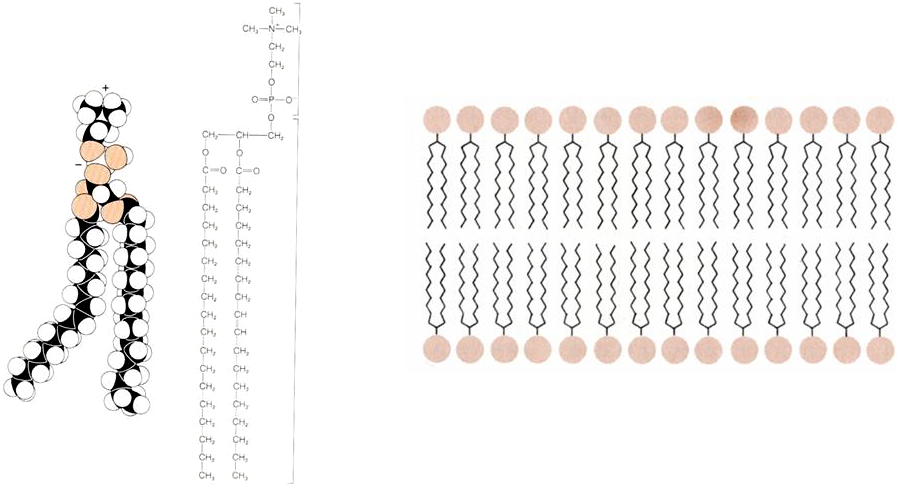
Diffusion is a surprisingly subtle phenomena that deserves some attention because its properties have dictated much of the physical structure of cells as well as the nervous system.

## Cell Membrane

The role of the cell membrane in distinguishing the intracellular fluid from the extracellular fluid is made possible by the fact that it is such an effective barrier to the transport of ions and polar molecules.

### Lipid Bilayer Structure

Cell membranes are composed of lipids and proteins. The predominant lipids in the cell membrane are **phospholipids**. Phospholipids have two distinct regions (Figure **4**). A polar region that is **hydrophilic** (water loving) that interacts with water molecules and a nonpolar region that is **hydrophobic** (water hating). Molecules that have a mixed chemical nature like this are known as **amphipathic** molecules.



##### **Figure 4** (Left panel) Structure of a phospholipid molecule. Note the charged head of the molecule and the two long hydrophobic tails. (right panel) Arrangement of phospholipids in a lipid bilayer, with the heads pointing out to the aqueous solution and the hydrophobic heads sequestered in the interior of the membrane.

The lipids assemble into a **lipid bilayer** (Figure **4**), which is the lowest energy arrangement for phospholipid molecules. In this arrangement the hydrophobic tails point in towards the center of the bilayer, minimizing their interaction with water molecules. The hydrophilic heads interact with the water molecules surrounding the membrane.

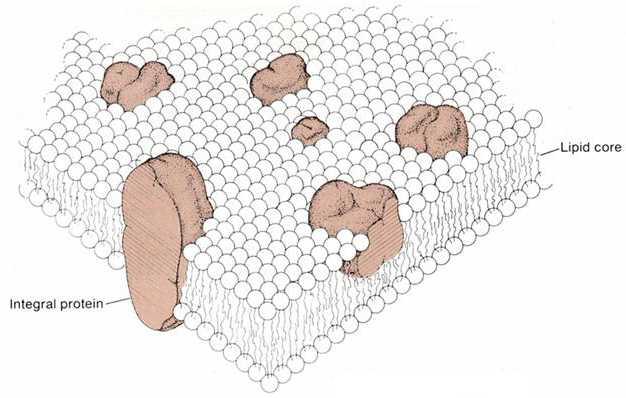
Although the lipid bilayer is very thin, it is a very effective barrier to the diffusion of many biologically important molecules. The interior of the lipid bilayer functions like a very thin layer of oil presenting an almost impermeable barrier to the diffusion of polar or charged molecules. In contrast, hydrophobic molecules can pass easily because they can dissolve into the hydrophobic core of the lipid bilayer.

Charged molecules like ions are only stable in a highly polarizable media such as water. It is essentially impossible for a charged molecule to cross the lipid bilayer because the core of the bilayer is non-polarizable. In electrical terms, it has a low dielectric constant.

If the cell membrane was composed only of a lipid bilayer only hydrophobic molecules could enter and leave the cell, which would greatly limit the function of the cell. Real cell membranes also contain proteins, and a major function of these proteins is to facilitate the movement of ions and polar molecules across the membrane.

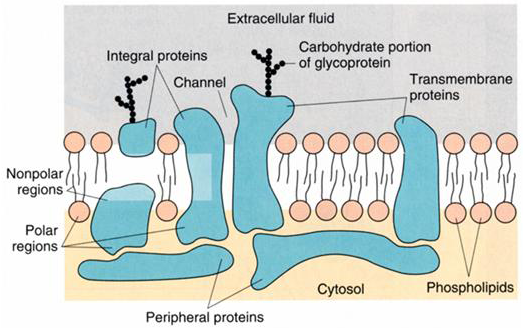
### Cell Membrane Structure

Current ideas about how the cell membrane functions originate with the fluid mosaic model of Singer and Nicolson. In their model a class of proteins, known as **integral membrane proteins**, are sequestered within the lipid bilayer, something like icebergs floating in a lipid sea (Figure 5). These proteins have amphipathic properties, meaning that they have both nonpolar portions, which are buried in the hydrocarbon core of the bilayer, and polar or charged portions, which protrude from the bilayer to form a hydrophilic surface that interacts with the aqueous phase. Typically, charged residues in the protein are only found in regions that contact the aqueous solution.



##### **Figure 5** Fluid mosaic model of the cell membrane, showing the integral proteins and the lipid bilayer.

In addition to integral membrane proteins there are also **peripheral membrane proteins**, which are typically located on the inner surface of the cell membrane (Figure 6). These are often linked to **cytoskeletal proteins**, which control cell shape and motility. In the original model, the integral membrane proteins were considered to move freely within the plane of the lipid bilayer. In fact, the majority of proteins are tethered to a dense network of other proteins, forming large protein complexes.



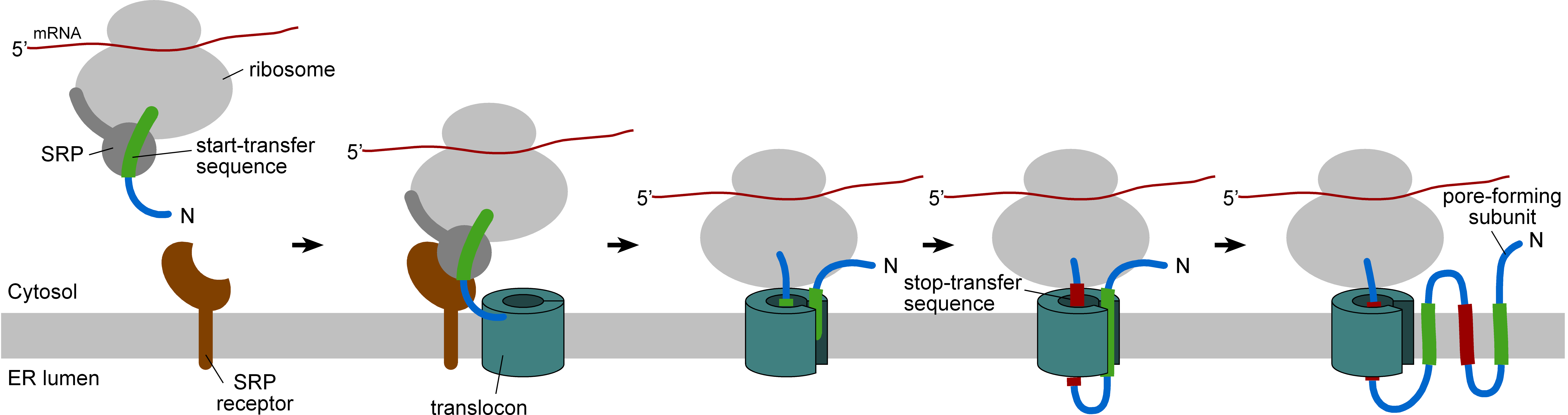
##### **Figure 6** Multiple types of proteins contribute to the formation of the cell membrane.

Typically, the **transmembrane proteins** (those that cross the cell membrane to the extracellular surface) have covalently linked carbohydrates on their extracellular surface (Figure 6) and are known as **glycoproteins**, a term that reflects the contribution of carbohydrate moieties to the protein structure.

### Synthesis of Membrane Proteins

Synthesis of integral membrane proteins, with one or more membrane spanning regions, is a complex problem for the cell, particularly achieving the correct topology of the protein in the membrane. Integral membrane proteins are synthesized by ribosomes that become tethered to a translocator complex (translocon) located in the endoplasmic reticulum (ER) membrane (**Figure 7**). Typically, the first transmembrane (TM) domain of the nascent peptide functions as a targeting sequence. This transmembrane sequence is recognized by the signal recognition particle (SRP), which in turn binds to the SRP receptor in the ER membrane. Following successful targeting to the ER membrane, the complex of ribosome and nascent peptide is transferred to the translocon. For proteins such as voltage-gated channels the amino terminus of the protein is located intracellularly and the first membrane spanning domain functions as a start-transfer signal, causing the translocon to mediate the translocation of the peptide trailing this transmembrane sequence through the translocon pore. This displaces the intralumenal plug that normally gates the translocon channel. Transfer of the peptide continues until the translocon encounters a stop-transfer signal (typically the second membrane spanning domain) causing the translocon to stop transferring the peptide across the membrane, thereby allowing the peptide to accumulate on the cytoplasmic side. In general, alternating start and stop-transfer signals in the protein’s peptide sequence will combine to allow the channel to assemble with the correct membrane topology. This topology signaling can include cues from regions of the polypeptide outside the transmembrane domain of the protein and more complex schemes may be required to ensure that proteins with non-canonical transmembrane domains, such as voltage sensors and channel pores, can achieve the correct topology.

In addition to having a trans-membrane pore for movement of the peptide across the membrane, the translocator complex is hinged and can open to allow the hydrophobic membrane spanning domains of the ion channel-forming protein to partition into the hydrophobic core of the membrane (**Figure 7**). By this means, the channel-forming protein is integrated into the lipid bilayer of the membrane.



##### **Figure 7** Most integral membrane proteins have hydrophobic transmembrane (TM) domains of 20–25 residues in length that form membrane spanning α-helices in the fully assembled protein (marked with red and green in the figure). The first of these TM domains acts as a targeting sequence to target the nascent peptide to the translocon in the ER membrane. This sequence is recognized by the signal recognition particle (SRP), which targets the entire complex to the ER membrane by binding to the SRP receptor. The ribosome and nascent peptide then attach directly to the translocon. The first TM domain is recognized as a start-transfer sequence by the translocon. This initiates movement of the downstream peptide through the translocon pore into the ER lumen. During subsequent translation of the protein the TM domains each signal to the translocon to start or stop transfer of the peptide across the ER membrane. The translocon has a lateral gate that can open to allow lateral transfer of each of the hydrophobic TM domains into the lipid bilayer.

Translocation of the growing peptide occurs co-translationally, meaning that the transfer of the protein into the ER lumen and membrane occurs at the same time as the protein is being synthesized. In general, the pore-forming subunit will first undergo further maturation, aided by proteins associated with the translocon complex such as oligosaccharyl transferase as well as by ER resident membrane chaperones (such as calnexin). Glycosylation of the channel peptide by oligosaccharyl transferase also occurs cotranslationally and can contribute to establishing the correct topology.

### Diffusion of Hydrophobic Molecules through the Lipid Bilayer

There are several ways in which a **solute** can either enter or leave the cell. If the solute is hydrophobic (lipophilic) and can dissolve into the lipid membrane, it can cross the cell membrane by diffusion since the lipid bilayer does not present a diffusion barrier. Many key molecules can act like this: oxygen, carbon dioxide, fatty acids and steroid hormones are all examples of nonpolar molecules that diffuse rapidly through the lipid portions of membranes.

The majority of molecules in the cell cannot diffuse through the membrane, or diffuse only poorly. For example, most of the molecules that make up the intermediate stages of the various metabolic pathways are ionized or polar molecules that cannot cross the cell membrane. There is a good reason for this, it is inefficient for the cell to expend energy producing metabolic intermediates that can then simply diffuse out of the cell in an uncontrolled manner. For the typical polar and charged molecules found inside the cell the lipid bilayer represents an almost complete barrier to passive diffusion.

## Membrane Transport

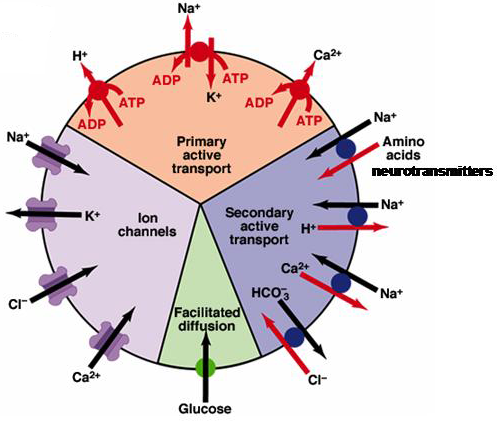
A diverse set of proteins facilitate the movement of polar and charged molecules in various ways across the cell membrane. These proteins fall into three major classes:

|  |  |
| --- | --- |
| Protein | Function |
| Pumps | require energy in the form of ATP to move ions up their concentration gradients |
| Transporters | do not directly use energy in the form of ATP, are often linked to ion gradients that indirectly provide energy |
| Ion channels | facilitate diffusion of ions by creating pores across the cell membrane |

##### **Table 2.** Classes of integral membrane proteins involved in transport of ions and polar molecules.

Figure 8 provides an overview of all the different mediated transport systems found in a typical cell membrane. Mediated transport implies the use of a protein molecule for the movement of a solute across the cell membrane, as opposed to simple diffusion of hydrophobic solutes through the lipid bilayer.

As noted, the Na,K-ATPase is arguably the single most important transport system in mammalian cells. It creates the nonequilibrium ion distributions, which produce electrical potential energy in the form of the membrane potential, so important in electrically excitable cells, and the sodium ion gradient, which provides the chemical energy source for the secondary active transporters. Only the facilitated diffusion systems function independently of the Na,K-ATPase pump.



##### **Figure 8** Summary of mediated membrane transport systems.

## Transport – Pumps

Most of the solutes distributed across the cell membrane are not in equilibrium. In particular, the major inorganic ions have steep distribution gradients across the cell membrane (Table 1). As a consequence, energy must be expended in order to maintain those transmembrane concentration gradients. Typically, the source of energy is chemical energy in the form of ATP. If the cell is poisoned so that ATP is no longer produced, then the transmembrane gradients dissipate and the cell dies.

The proteins that actively transport solutes against their concentration gradients are known as membrane pumps. Four pumps have been identified, each is an ATPase and each is involved in transporting one or more of the following ions: Na+, K+, H+ or Ca2+.

|  |  |
| --- | --- |
| Pump | Function |
| Na,K-ATPase | maintains the Na+, K+ ion gradients across the cell membrane |
| Ca-ATPase | maintains the very low intracellular Ca2+ ion concentration |
| H-ATPase | maintains intracellular pH (H+ ion concentration) |
| H,K-ATPase | acid secretion in stomach and kidneys |

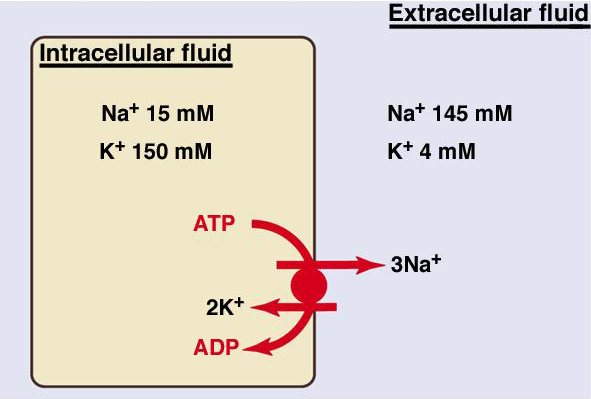
##### **Table 3.** Types of membrane pumps.

### Na,K-ATPase

An argument can be made that the Na,K-ATPase pump is the most important protein in the cell because, in most cells, it is the single largest consumer of cellular energy. Surprisingly, the maintenance of ion gradients is the most energetically demanding process within many cells. More expensive than many other apparently more sophisticated functions, such as protein synthesis.

The Na,K-ATPase pump actively pumps Na+ ions out of the cell against their concentration gradient. It simultaneously pumps K+ ions into the cell and the metabolic energy for both of these functions is provided by the hydrolysis of ATP to ADP (Figure 9). Two K+ ions from outside the cell are exchanged for three Na+ ions from inside the cell.

The pump is known as an **electrogenic** pump because there is an associated electrical current, the net movement of one positive ion out of the cell for every cycle of the pump. This current is generally very small relative to other ion currents in electrically excitable cells.



##### **Figure 9** Movement of metabolites and ions during one cycle of the Na,K-ATPase pump .

The Na,K-ATPase is present in the plasma membranes of all animal cells. It is responsible for the fact that all animal cells have a relatively high intracellular concentration of potassium ions and a low intracellular concentration of sodium ions.

The pump can be poisoned or inhibited with the drug **ouabain**, which ultimately results in the death of the cell. Ouabain was originally isolated from plants in Africa and was used to poison arrow tips. Surprisingly, similar compounds are used in modern medicine to block the pump in the treatment of some forms of congestive heart failure.

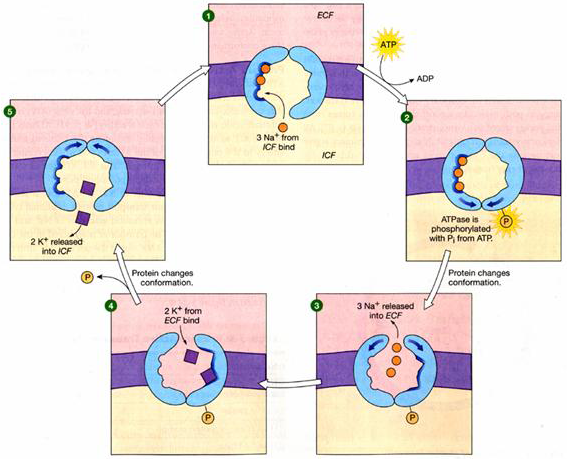
### Other pumps

The Ca-ATPase is found in both the cell membrane and in the membrane of several organelles within the cell. As shown in Table 1, the internal concentration of Ca2+ ions is very low compared with the extracellular Ca2+ ion concentration, in large part due to active pumping of Ca2+ ions out of the cell. There is an approximately 1:20,000 gradient in Ca2+ ion concentration gradient between the interior of the cell and the extracellular fluid.

The other two pumps move hydrogen ions, controlling intracellular or extracellular acidity. The H-ATPase helps regulate intracellular pH in all cells. The H,K-ATPase is involved in acid secretion by specialized cells in the stomach and kidneys.

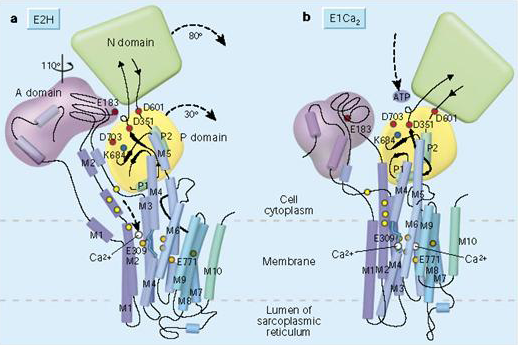
### Structure and Function of Membrane Pumps

All four pumps work similarly and have similar structures. They function by translocating an ion binding surface from inside the cell to outside the cell and then a modified surface from outside back to the inside of the cell. The movement of this surface is produced by a **conformational change** in the shape of the protein. The hydrolysis of ATP provides the energy for this change in protein shape. The kinase function of the pump is used to phosphorylate the pump protein, which then induces the first conformational change (Figure 10). Dephosphorylation of the pump, to remove the covalently linked phosphate group, results in the reversion of the pump to its original conformation.



##### **Figure 10** Cycle of Na+ and K+ binding and movement during one cycle of the Na,K-ATPase pump.

The detailed structure of the Ca-ATPase pump has been solved. It is a large protein and has ten membrane spanning domains (Figure 11). There are two binding sites for Ca2+ ions within the membrane and it has a very large intracellular domain, which contains the ATPase enzyme that hydrolyzes ATP. The pump undergoes large rearrangements upon phosphorylation and dephosphorylation of the ATPase site (Figure 11). This results in the rearrangement of the alpha helices in the membrane so that the Ca2+ binding sites are moved from facing the intracellular region of the membrane to facing the extracellular region and also causes a reduction of the affinity for Ca2+ ion binding so that the ions are released into the extracellular fluid or the interior of membrane bound organelles.



##### **Figure 11** Conformational changes of the Ca-ATPase before and after phosphorylation.

This requirement for a large conformational change limits the rate at which ion pumps can move ions across the membrane. In general, the pumps are continuously active in order to keep up with the flux of ions through the membrane’s ion channels, which allow ions to move very rapidly down their ion concentration gradients. In most cells the ion channels turn on for only brief periods of time in order to limit the amount of work required of the pumps. An exception to this is found in cardiac myocytes, where the Ca2+ pumps have to return Ca2+ ions back to the lumen of the sarcoplasmic reticulum after it has escaped through Ca2+ channels that remain open for the duration of the cardiac contraction. In this case, very high concentrations of the Ca-ATPase pump are required in the SR membrane in order to keep up with the calcium release and this protein makes up a large fraction of the total membrane protein in cardiac cells. This is one reason why we are so vulnerable to ischemia during a heart attack. When blood flow stops even for a short period of time there can be significant damage to the cardiac muscle because it fails to meet the energy demands of the Ca-ATPase pump.

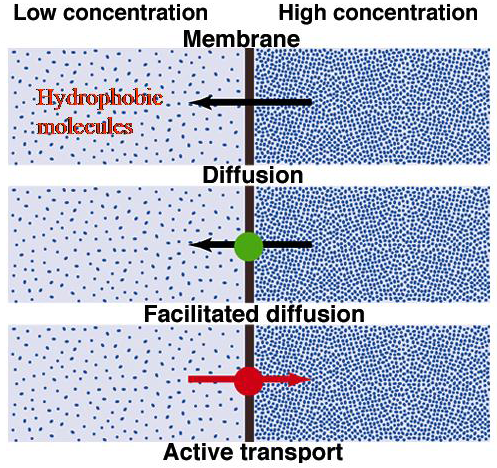
### Ion Gradients as Sources of Cellular Energy

The generation of ion gradients by pumps is one way in which the cell can convert chemical energy, stored in the form of ATP, into another form of chemical energy, in this case a concentration gradient of ions. The gradient of ions acts as a source of chemical energy that can be used for other cellular functions such as **secondary active transport**.

The generation of ion gradients can also function to convert chemical energy into electrical energy. The ion gradients created by the pumps allow the generation of an electrical potential across the cell membrane, known as the **membrane potential**.

## Membrane Transport - Transporters

Pumps and ion channels only move ions across the cell membrane. All cells, however, have to transport a large number of solutes in addition to ions. These solutes belong to a diverse set of biochemical molecules that are useful to the cell, including amino acids and glucose. In general, the different chemical natures of these solutes requires that there are specialized transport systems for each type, or at least class, of molecule that is transported. There are two types of transport systems, those that facilitate the movement of solutes down their concentration gradients and those that actively transport the solute up a concentration gradient (Figure 12). As mentioned earlier, hydrophobic molecules can move freely cross the membrane without the requirement of a specific transport system. It would be self-defeating to actively transport these molecules since their movement is not limited by the cell membrane and they could easily diffuse back across the membrane.

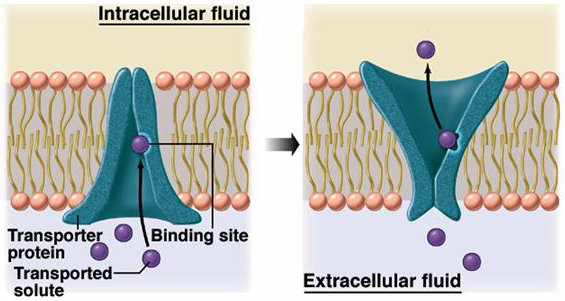


##### **Figure 12** Hydrophobic molecules can diffuse down their concentration gradient without any specialized transport system. Facilitated diffusion transport systems allow the movement of hydrophilic solutes down their concentration gradients. Active transport can move hydrophilic solutes across the membrane up a concentration gradient.

### Facilitated Diffusion

The simplest of all the membrane transport mechanisms is known as **facilitated diffusion**. It does not require any energy input. Solutes simply diffuse down their concentration gradients. The facilitated transporter proteins provide a wrapper to allow hydrophilic molecules to pass across the cell membrane by avoiding contact with the lipid bilayer (Figure 13).

An important example of facilitated transport is the **passive glucose transporter**, which is found in the membrane of most cells. This transporter facilitates the movement of glucose from the blood stream into the cells of most organs, where it is used as an energy source. A transporter is required because glucose is a polar molecule that cannot otherwise cross the cell membrane. Without this transporter most cells would not be able to use the glucose produced by the breakdown of ingested food.



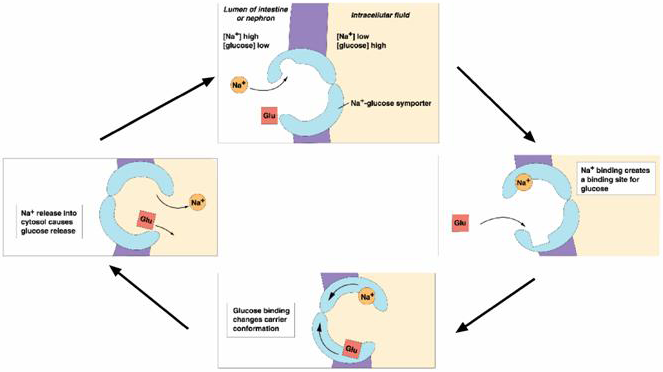
##### **Figure 13** The solute binding site of a facilitated transporter flips from one side of the membrane to the other allowing solutes to move down their concentration gradient.

### Secondary Active Transport

The gradient of sodium ions across the cell membrane generated by the Na,K-ATPase pump is a form of stored energy. This energy can be used by transport proteins to move solutes up their concentration gradients. These transport proteins are known as **secondary active transporters**. They are called secondary because they use chemical energy stored in the form of an ion gradient rather than directly use ATP. They are active transporters, like pumps, in that they move solutes up a concentration gradient.

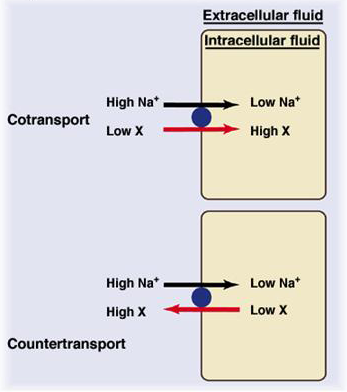
An important example of a secondary active transporter is the **Na+/glucose transporter** (Figure 14). In addition to having a binding site for glucose the glucose transporter has an additional binding site for a Na+ ion. The binding of the Na+ ion to the transporter alters the affinity of the binding site for glucose. The change is brought about through allosteric modification of the protein’s conformation as a result of ion binding. One glucose molecule is translocated up its concentration gradient at the cost of one Na+ ion moving down its concentration gradient.

The Na+/glucose transporter is used to actively transport glucose out of the intestines and into the blood stream and also out of the kidney tubules and back into the blood.



##### **Figure 14** Na+/glucose transporter.

During secondary active transport a solute can be transported either into the cell (**cotransport**) or out of the cell (**countertransport**). In both cases, however, Na+ ions move into the cell, down their concentration gradient (Figure 15).

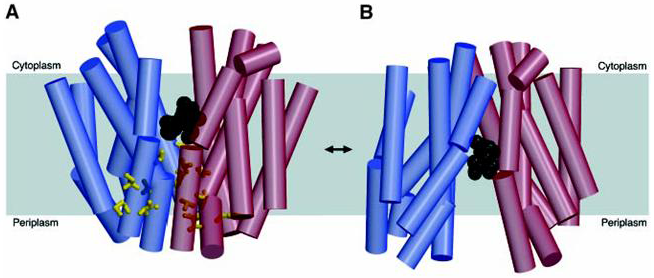


##### **Figure 15** Cotransport and countertransport.

Another important secondary active transporter is the Na-Ca countertransporter, or **Na-Ca exchanger**. This uses downhill movement of sodium ions into the cell to move calcium ions out of the cell by secondary active transport. Other transporters linked to the sodium ion gradient move **amino acids**. Amino acids can be actively transported out of the kidney tubules and into the blood by sodium-driven transporters. Related transporters mediate the reuptake of some neurotransmitters from the synaptic cleft in the nervous system.

### Structure of Secondary Active Transporters

The LacY transporter is the prototype for transporter proteins. The LacY transporter mediates the coupled cotransport of lactose and protons (H+) down a proton gradient in prokaryotyes. It has a roughly symmetrical clamshell-like structure with twelve membrane spanning domains (Figure 16).

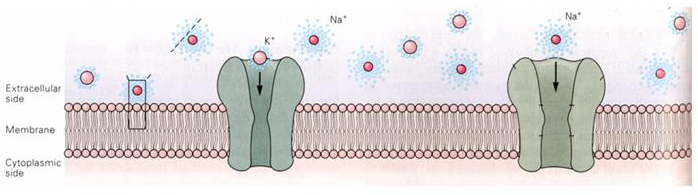


##### **Figure 16** Structure of lactose permease (LacY). Note the two different conformations of the transporter

Relatively large movements of the protein are required to produce the translocation of the solute binding site. Like pumps, active transporters move solutes slowly compared to the movement of ions through ion channels.

## Membrane Transport - Ion channels

The function of ion channel proteins is captured almost perfectly by their name, they are channels through which ions can pass across the membrane. Ion channels are integral membrane proteins and they shield the charged ions from the hydrophobic lipid bilayer as the ions cross the cell membrane (Figure 17). One key feature of ion channels is that they show **ion selectivity**. There are channels that only let K+ ions to pass and channels that only let Na+ ions to pass.



##### **Figure 17** A K+ selective ion channel and a Na+ selective ion channel in the cell membrane.

At rest, in a typical cell, only a small number of channels are open and available to pass ions at any one time. Collectively, the channels open in a resting cell are known as the **leak channels** and these leak channels are predominantly K+ selective with few or no Na+ channels open. As a consequence, at rest, the cell membrane is predominantly permeable to K+ ions.

Ion channels are found in the cell membrane of all cells in the human body and in the cell membranes of almost all living organisms, and many viruses. Ion channels are of particular interest in electrically excitable cells because of their key role in generating electrical excitation. The other two types of transporters are also ubiquitous and create the basic cellular environment necessary to support electrical activity, as well as many other cell functions. The structure and function of ion channels will be described in detail in Chapter 2.

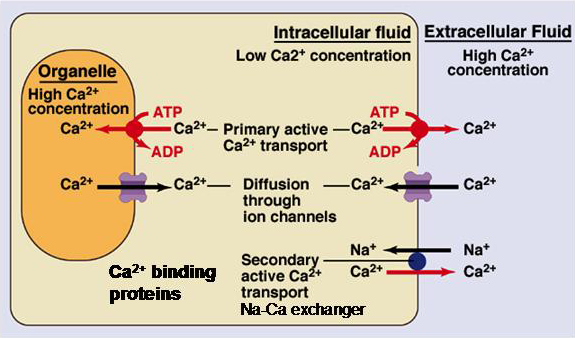
## Regulation of Internal Ca2+ Ion Concentration

Intracellular Ca2+ ion concentrations are maintained at very low levels inside the cell and there is a very large concentration gradient of Ca2+ ions across the cell membrane (Table 4).

|  |  |  |
| --- | --- | --- |
| Ion | Intracellular | Extracellular |
| Ca2+ | 100 nM | 2 mM |

##### **Table 4.** Calcium ion concentrations inside and outside of cell

There are a multitude of transport systems that contribute to the regulation of the internal Ca2+ ion concentration (Figure 18). The Ca-ATPase pump is found in the cell membrane and several organelle membranes. It actively pumps Ca2+ ions out of the cell or into intracellular organelles (mitochondria and endoplasmic reticulum), where the Ca2+ ions are sequestered. In addition, there is the Na-Ca exchanger that uses the energy of the Na+ ion gradient to transport Ca2+ ions out of the cell. Calcium binding proteins are another important part of the system. These proteins bind Ca2+ ions and act like buffers, rapidly reducing Ca2+ ion concentrations following an influx of Ca2+ ions through open calcium channels. These buffers give the more slowly acting pump and transporters some time to remove the ions from the intracellular fluid. The buffers limit the duration and extent of the change in free Ca2+ ion concentration within the cell.



##### **Figure 18** Summary of the various systems for handling Ca2+ ions within the cell.

### Ca2+ Ions as Second Messengers

Perhaps oddly, given the effort that cells expend in keeping internal Ca2+ ion concentrations low, Ca2+ ions have an important role as intracellular signaling molecules. Although an apparently unlikely candidate, given the simple nature of these molecules, Ca2+ ions modulate the function of a myriad of proteins and a wide variety of cellular functions are sensitive to changes in intracellular Ca2+ ion concentrations.

Typically, increases in Ca2+ ion concentrations are triggered by the opening of Ca2+ channels, either in the cell membrane or in the membranes of the organelles that sequester Ca2+ ions. These calcium fluxes produce a transient increase in the free calcium ion concentration in the cell that triggers downstream cell signaling pathways. This signaling system is used by all cells but is particularly important for electrically excitable cells because it provides a means of converting an electrical signal into a biochemical one. Examples of important cellular functions dependent on Ca2+ signaling include synaptic transmission and muscle contraction.

### Internal Ca2+ Concentration and Cell Death

Maintenance of a low intracellular Ca2+ ion concentration is critical for normal cell function. In most cells, a prolonged increase in intracellular Ca2+ ion concentrations rapidly leads to cell death.

Blockade of blood flow (and thus oxygen) in the brain or the heart quickly leads to **ischemic tissue damage** in these organs. The brain and the heart are very metabolically active tissues and as a consequence use up their local energy supplies very quickly. This makes them particularly vulnerable to ischemic tissue damage because the maintenance of low Ca2+ ion concentrations inside the cell is strongly dependent on maintained cellular energy levels. The decrease in local oxygen tension during ischemia results in a rapid fall in ATP levels inside the cell, which leads to a rise in calcium levels. The rise in intracellular calcium levels can trigger cellular processes that lead to the destruction of the cell.

## Maintenance of Cell Volume

The most abundant molecules both inside the cell and in the extracellular solution are water molecules, which make the major contribution to the volume of the intracellular and extracellular solutions. As a consequence, the flow of water into or out of the cell across the cell membrane is the primary determinant of changes in cell volume.

### Water Channels

The cell membrane is highly permeant to water molecules. For much of the history of cellular physiology the high permeability of the cell membrane to water was something of a mystery because H2O is a highly polar molecule that cannot easily cross the lipid bilayer. It was ultimately determined that there are specialized membrane transport proteins for water molecules known as **water channels** (or **aquaporins**).

Water channels are integral membrane proteins, analogous to ion channels, that provide a low resistance pathway for the movement of water molecules across the cell membrane.

### Osmolarity

Osmolarity is a measure of the concentration of osmotically active particles in a solution, typically expressed as osmoles of solute per liter of solution. For molecules such as glucose, sucrose and urea that do not dissociate, a solution containing 1 mole of dissolved molecules in 1 liter of water is a 1 osmole/liter solution. For salts or acids dissolved in solution the situation is slightly more complex because these compounds dissociate into two or more ions in solution. For NaCl, which dissociates into two dissolved particles, the Na+ and Cl- ions, a 1M NaCl solution is a 2 osmole/l solution. For CaCl2, which dissociates into three ions, a 1M CaCl2 solution is a 3 osmole/l solution.

### Regulation of Cell Volume

Maintaining a balance between the osmolarity inside the cell versus the osmolarity of the extracellular solution is critical to maintain the integrity of the cell membrane. To limit potential damage to the cell membrane, the osmolarity of extracellular solution is kept within relatively tight limits, in the range 275-295 mosmole/l in mammals.

To understand the effect of changes in intracellular or extracellular osmolarity on cell volume it is important to recognize that water has a concentration (number of molecules per unit volume) just like the solutes dissolved in a solution. The concentration of H2O molecules in pure water is approximately 55.5M. If sugar molecules are dissolved into water the volume of the resulting solution increases because the sugar molecules take up some volume in the solution. Assuming that each solute molecule takes up the space of one water molecule, for a 1 M glucose solution, the water concentration falls to approximately 54.5M, significantly less that the 55.5M value for pure water. As a consequence, the concentration of water molecules in a sugar solution is lower than it is in pure water.

Water can flow down its concentration gradient across the cell membrane, just like membrane permeable solutes. If the concentration of water outside of a cell is higher than it is inside the cell, water will flow into the cell across the cell membrane until the concentration of water is equal on each side of the membrane. An extreme example of this is if a cell is placed in distilled water (water containing no ions or other solvents). In this case the cell rapidly expands and dies, because the osmolarity inside the cell is much higher than outside and water flows rapidly into the cell, down its concentration gradient.