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Introducción a la Biología Celular

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PROTEÍNAS

Las proteínas de membrana dan cuenta de la mayoría de las funciones específicas de las membranas. Las proteínas se asocian con la bicapa de varias maneras distintas.

Various ways in which membrane proteins associate with the lipid bilayer. Most trans-membrane proteins are thought to extend across the bilayer as (1) a single α helix, (2) as multiple α helices, or (3) as a rolled-up β sheet (a β barrel). Some of these "single-pass" and "multipass" proteins have a covalently attached fatty acid chain inserted in the cytosolic lipid monolayer (1). Other membrane proteins are exposed at only one side of the membrane. (4) Some of these are anchored to the cytosolic surface by an amphipathic α helix that partitions into the cytosolic monolayer of the lipid bilayer through the hydrophobic face of the helix. (5) Others are attached to the bilayer solely by a covalently attached lipid chain— either a fatty acid chain or a prenyl group—in the cytosolic monolayer or, (6) via an oligosaccharide linker, to phosphatidylinositol in the noncytosolic monolayer. (7, 8) Finally, many proteins are attached to the membrane only by noncovalent interactions with other membrane proteins.

Known structures for membrane proteins **a**, Proteins largely within the membrane bilayer. **b**, Proteins with large extramembrane regions. **c**, Proteins covering large regions of lipid. The green indicates amino acids with a favourable interaction with the hydrophobic lipid region, blue a favourable interaction with water. (Engelman DM 2005 *Nature* **438**: 578-580)

A segment of a transmembrane polypeptide chain crossing the lipid bilayer as an a helix. Only the a-carbon backbone of the polypeptide chain is shown, with the hydrophobic amino acids in *green* and *yellow*. The polypeptide segment shown is part of the bacterial photo-synthetic reaction center illustrated in Figure 10-38, the structure of which was determined by x-ray diffraction. (Based on data from J. Deisenhofer et al., *Nature* 318:618-624, 1985, and H. Michel et al., *EMBO J.* 5:1149-1158, 1986.)

Hydropathy plots and potential α-helical membrane-spanning segments in a polypeptide chain. Free energy needed to transfer successive segments of a polypeptide chain from a nonpolar solvent to water is calculated from the amino acid composition of each segment using data obtained with model compounds. This calculation is made for segments of a fixed size. A positive value indicates that free energy is required for transfer to water. Peaks in the hydropathy index appear at the positions of hydrophobic segments in the amino acid sequence. (A and B) Glycophorin (A) has a single membrane-spanning α helix and one corresponding peak in the hydropathy plot. Bacteriorhodopsin (B) has seven membrane-spanning α helices and seven corresponding peaks in the hydropathy plot. (C) The proportion of predicted membrane proteins in the genomes of *E. coli, S. cerevisiae,* and human. The curves for *E. coli* and *S. cerevisiae* represent the whole genome.

Two α-helices in the aquaporin water chanel, each of which spans only halfway through the lipid bilayer

Converting a single chain multipass protein into a two chain multipass protein

β barrels formed from different numbers of β strands. (1) The *E. coli* OmpA protein (8 β strands), which serves as a receptor for a bacterial virus. (2) The *E. coli* OMPLA protein (12 β strands), is a lipase that hydrolyses lipid molecules. The amino acids that catalyze the enzymatic reaction (shown in *red*) protrude from the outside surface of the barrel. (3) A porin from the bacterium *Rhodobacter capsulatus,* which forms water-filled pores across the outer membrane (16 β strands). The diameter of the channel is restricted by loops (shown in *blue*) that protrude into the channel. (4) The *E. coli* FepA protein (22 β strands), which transports iron ions. The inside of the barrel is completely filled by a globular protein domain (shown in *blue*) that contains an iron-binding site. This domain is thought to change its conformation to transport the bound iron, but the molecular details of the changes are not known.

A single-pass transmembrane protein. Note that the polypeptide chain traverses the lipid bilayer as a right-handed a helix and that the oligosaccharide chains and disulfide bonds are all on the noncytosolic surface of the membrane. Disulfide bonds do not form between the sulfhydryl groups in the cytoplasmic domain of the protein because the reducing environment in the cytosol maintains these groups in their reduced (-SH) form.

The three-dimensional structure of a bacteriorhodopsin molecule. The polypeptide chain crosses the lipid bilayer seven times as α helices. The location of the retinal chromophore *(purple)* and the probable pathway taken by protons during the light-activated pumping cycle are shown. The first and key step is the passing of a H^{+} from the chromophore to the side chain of aspartic acid 85 *(red,* located next to the chromophore) that occurs upon absorption of a photon by the chromophore. Subsequently, other H^{+} transfers—utilizing the hydrophilic amino acid side chains that line a path through the membrane—complete the pumping cycle and return the enzyme to its starting state. Color code: glutamic acid *(orange),* aspartic acid *(red),* arginine *(blue).* (Adapted from H. Luecke et al., *Science* 286:255–260, 1999.)

The three-dimensional structure of the photosynthetic reaction center of the bacterium *Rhodopseudomonas viridis*. The structure was determined by x-ray diffraction analysis of crystals of this transmembrane protein complex. The complex consists of four subunits L, M, H, and a cytochrome. The L and M subunits form the core of the reaction center, and each contains five α helices that span the lipid bilayer. The locations of the various electron carrier coenzymes are shown in *black*. Note that the coenzymes are arranged in the spaces between the helices. (Adapted from a drawing by J. Richardson based on data from J. Deisenhofer, O. Epp, K. Miki, R. Huber, and H. Michel, *Nature* 318:618–624, 1985.)

The three-dimensional structure of a porin trimer of *Rhodobacter capsulatus* determined by x-ray crystallography. (A) Each monomer consists of a 16-stranded antiparallel b barrel that forms a transmembrane water-filled channel. (B) The monomers tightly associate to form trimers, which have three separate channels for the diffusion of small solutes through the bacterial outer membrane. A long loop of polypeptide chain (shown in *red*), which connects two b strands, protrudes into the lumen of each channel, narrowing it to a cross-section of 0.6 x 1 nm. (Adapted from M.S. Weiss et al., *FEBS Lett.*280: 379-382, 1991.)

Muchas de las proteínas de membrana, en especial las de la membrana plasmática, están glicosiladas.

Las proteínas de membrana se pueden solubilizar y purificar en detergentes. Las proteínas así purificadas se pueden re-insertar en liposomas para estudiar su actividad.

A detergent micelle in water, shown in cross section. Because they have both polar and nonpolar ends, detergent molecules are amphipathic. Because they are wedge-shaped, they form micelles rather than bilayers

Solubilizing membrane proteins with a mild detergent. The detergent disrupts the lipid bilayer and brings the proteins into solution as protein-lipid-detergent complexes. The phospholipids in the membrane are also solubilized by the detergent.

The structures of two commonly used detergents. Sodium dodecyl sulfate (SDS) is an anionic detergent, and Triton X-100 is a nonionic detergent. The hydrophobic portion of each detergent is shown in *green,* and the hydrophilic portion is shown in *blue.* The bracketed portion of Triton X-100 is repeated about eight times.
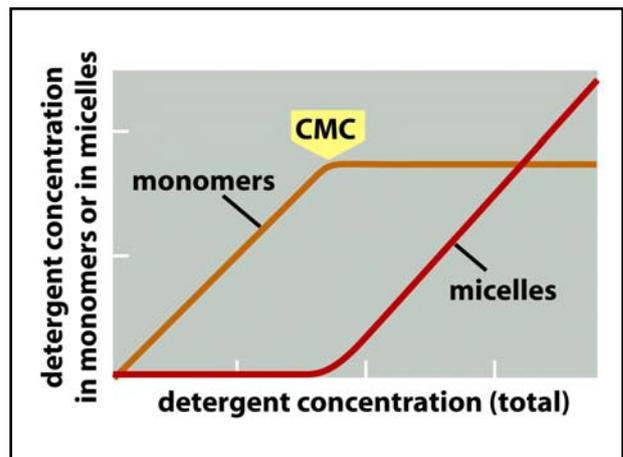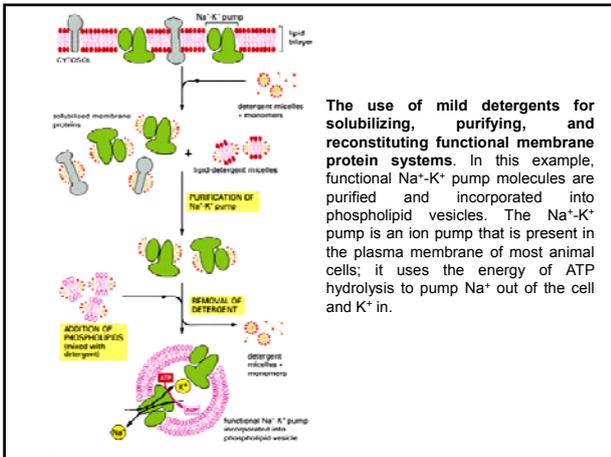
The use of mild detergents for solubilizing, purifying, and reconstituting functional membrane protein systems. In this example, functional Na$^+$-K$^+$ pump molecules are purified and incorporated into phospholipid vesicles. The Na$^+$-K$^+$ pump is an ion pump that is present in the plasma membrane of most animal cells; it uses the energy of ATP hydrolysis to pump Na$^+$ out of the cell and K$^+$ in.
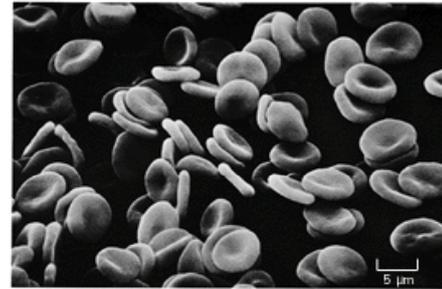
A scanning electron micrograph of human red blood cells. The cells have a biconcave shape and lack nuclei. (Courtesy of Bernadette Chailley.)
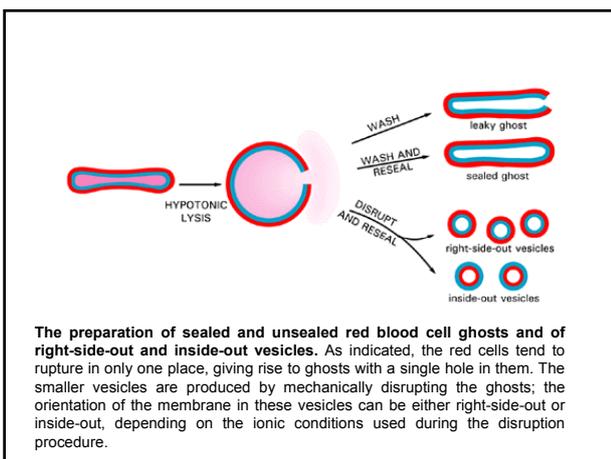
The preparation of sealed and unsealed red blood cell ghosts and of right-side-out and inside-out vesicles. As indicated, the red cells tend to rupture in only one place, giving rise to ghosts with a single hole in them. The smaller vesicles are produced by mechanically disrupting the ghosts; the orientation of the membrane in these vesicles can be either right-side-out or inside-out, depending on the ionic conditions used during the disruption procedure.

Muchas de las proteínas de membrana difunden (se mueven) en el plano lateral de la membrana. No obstante, existen mecanismos que pueden confinar proteínas y lípidos (rafts) en dominios específicos de la membrana.

Experiment demonstrating the mixing of plasma membrane proteins on mouse-human hybrid cells. The mouse and human proteins are initially confined to their own halves of the newly formed heterocaryon plasma membrane, but they intermix with time. The two antibodies used to visualize the proteins can be distinguished in a fluorescence microscope because fluorescein is green whereas rhodamine is red. (Based on observations of L.D. Frye and M. Edidin, *J. Cell Sci.* 7:319-335, 1970, by permission of The Company of Biologists.)

Measuring the rate of lateral diffusion of a plasma membrane protein by the FRAP technique. A specific protein is labeled on the cell surface with a fluorescent monovalent antibody that binds only to that protein (for simplicity, no other proteins are shown). After the antibodies are bleached in a small area using a laser beam, the fluorescence intensity recovers as the bleached molecules diffuse away and unbleached molecules diffuse into the irradiated area (shown in side view in A and top view in B). (C) A graph showing the rate of recovery. The greater the diffusion coefficient of the membrane protein, the faster the recovery.

Measuring the rate of lateral diffusion of a membrane protein by photobleaching techniques. A specific protein of interest can be labeled with a fluorescent antibody (as shown here), or it can be expressed as a fusion protein with green fluorescent protein (GFP), which is intrinsically fluorescent. In the FRAP technique, fluorescent molecules are bleached in a small area using a laser beam. The fluorescence intensity recovers as the bleached molecules diffuse away and unbleached molecules diffuse into the irradiated area (shown here in side and top views). The diffusion coefficient is calculated from a graph of the rate of recovery: the greater the diffusion coefficient of the membrane protein, the faster the recovery.

Measuring the rate of lateral diffusion of a membrane protein by photobleaching techniques. In the FLIP technique, an area in the membrane is irradiated continuously, and fluorescence is measured in a separate area. Fluorescence in the second area progressively decreases as fluorescent proteins diffuse out and bleached molecules diffuse in; eventually, all of the fluorescent protein molecules will be bleached, as long as they are mobile and not permanently anchored to the cytoskeleton or extracellular matrix.

How a plasma membrane protein is restricted to a particular membrane domain. In this drawing of an epithelial cell, protein A (in the apical membrane) and protein B (in the basal and lateral membranes) can diffuse laterally in their own domains but are prevented from entering the other domain, at least partly by the specialized cell junction called a tight junction. Lipid molecules in the outer (noncytosolic) monolayer of the plasma membrane are likewise unable to diffuse between the two domains; lipids in the inner (cytosolic) monolayer, however, are able to do so (not shown).
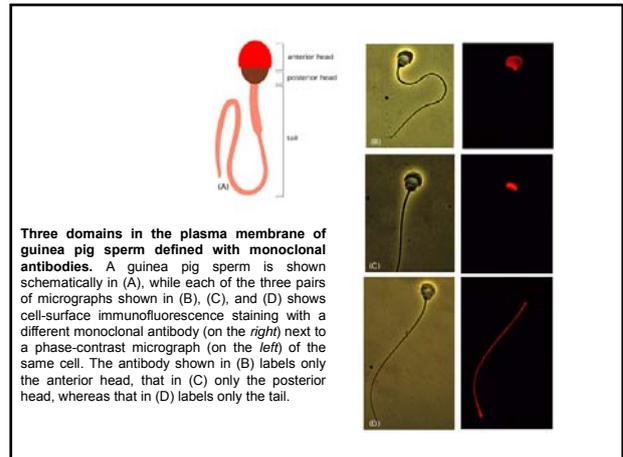
Three domains in the plasma membrane of guinea pig sperm defined with monoclonal antibodies. A guinea pig sperm is shown schematically in (A), while each of the three pairs of micrographs shown in (B), (C), and (D) shows cell-surface immunofluorescence staining with a different monoclonal antibody (on the *right*) next to a phase-contrast micrograph (on the *left*) of the same cell. The antibody shown in (B) labels only the anterior head, that in (C) only the posterior head, whereas that in (D) labels only the tail.
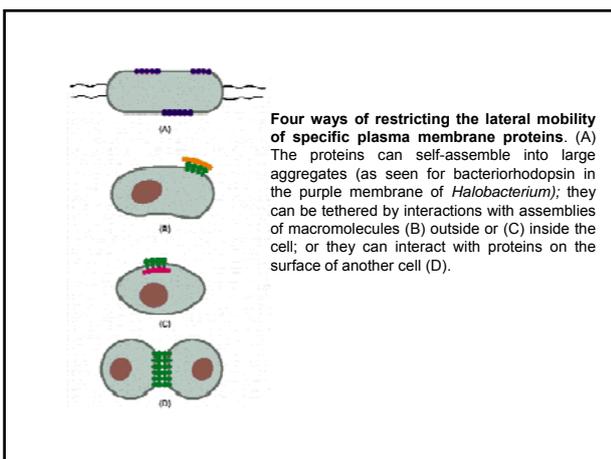
Four ways of restricting the lateral mobility of specific plasma membrane proteins. (A) The proteins can self-assemble into large aggregates (as seen for bacteriorhodopsin in the purple membrane of *Halobacterium);* they can be tethered by interactions with assemblies of macromolecules (B) outside or (C) inside the cell; or they can interact with proteins on the surface of another cell (D).
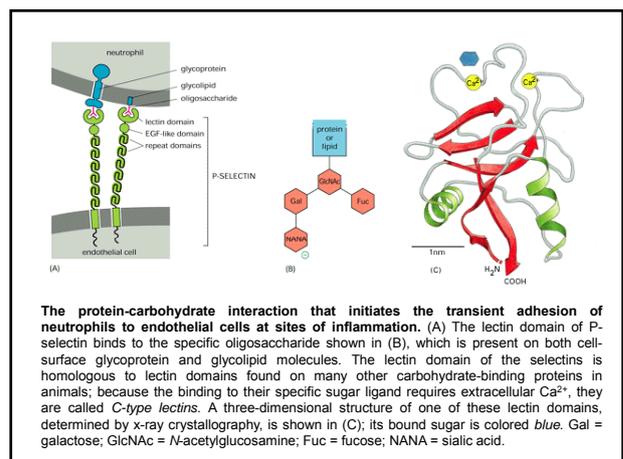
The protein-carbohydrate interaction that initiates the transient adhesion of neutrophils to endothelial cells at sites of inflammation. (A) The lectin domain of P-selectin binds to the specific oligosaccharide shown in (B), which is present on both cell-surface glycoprotein and glycolipid molecules. The lectin domain of the selectins is homologous to lectin domains found on many other carbohydrate-binding proteins in animals; because the binding to their specific sugar ligand requires extracellular Ca^{2+}, they are called *C-type lectins*. A three-dimensional structure of one of these lectin domains, determined by x-ray crystallography, is shown in (C); its bound sugar is colored *blue*. Gal = galactose; GlcNAc = *N*-acetylglucosamine; Fuc = fucose; NANA = sialic acid.

TRANSPORTE

Uniport | Symport | Antiport
Cotransport
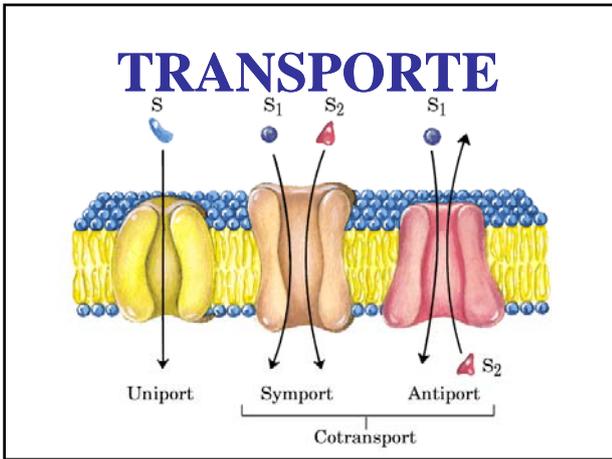
A Comparison of Ion Concentrations Inside and Outside a Typical Mammalian Cell

COMPONENT	INTRACELLULAR (mM)	EXTRACELLULAR (mM)
Cations		
Na^+	5-15	145
K^+	140	5
Mg^{2+}	0.5	1-2
Ca^{2+}	10^{-4}	1-2
H^+	7×10^{-5} ($10^{-7.2}$ M or pH 7.2)	4×10^{-5} ($10^{-7.4}$ M or pH 7.4)
Anions*		
Cl^-	5-15	110

* The cell must contain equal quantities of positive and negative charges (that is, be electrically neutral). Thus, in addition to Cl^-, the cell contains many other anions not listed in this table; in fact, most cellular constituents are negatively charged (HCO_3^-, PO_4^{3-}, proteins, nucleic acids, metabolites carrying phosphate and carboxyl groups, etc.). The concentrations of Ca^{2+} and Mg^{2+} given are for the free ions. There is a total of about 20 mM Mg^{2+} and 1-2 mM Ca^{2+} in cells, but this is mostly bound to proteins and other substances and, for Ca^{2+}, stored within various organelles.

Las bicapas lipídicas son notablemente impermeables a los iones

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The relative permeability of a synthetic lipid bilayer to different classes of molecules. The smaller the molecule and, more importantly, the less strongly it associates with water, the more rapidly the molecule diffuses across the bilayer.

Permeability coefficients for the passage of various molecules through synthetic lipid bilayers. The rate of flow of a solute across the bilayer is directly proportional to the difference in its concentration on the two sides of the membrane. Multiplying this concentration difference (in mol/cm^3) by the permeability coefficient (in cm/sec) gives the flow of solute in moles per second per square centimeter of membrane. A concentration difference of tryptophan of 10^{-4} mol/cm^3 ($10^{-4}/10^{-3}$ L = 0.1 M), for example, would cause a flow of 10^{-4} $mol/cm^3 \times 10^{-7}$ cm/sec = 10^{-11} mol/sec through 1 cm^2 of membrane, or 6×10^4 molecules/sec through 1 μm^2 of membrane.

Existen dos clases principales de proteínas de membrana que dan cuenta de los fenómenos de transporte: las proteínas transportadoras (*carriers*) y los canales (*channels*). El transporte puede ser pasivo o activo

Carrier proteins and channel proteins. (A) A carrier protein alternates between two conformations, so that the solute-binding site is sequentially accessible on one side of the bilayer and then on the other. (B) In contrast, a channel protein forms a water-filled pore across the bilayer through which specific solutes can diffuse

Passive and active transport compared. (A) Passive transport down an electrochemical gradient occurs spontaneously, either by simple diffusion through the lipid bilayer or by facilitated diffusion through channels and passive carriers. By contrast, active transport requires an input of metabolic energy and is always mediated by carriers that harvest metabolic energy to pump the solute against its electrochemical gradient. (B) An electrochemical gradient combines the membrane potential and the concentration gradient, which can work additively to increase the driving force on an ion across the membrane *(middle)* or can work against each other *(right)*.

A model of how a conformational change in a carrier protein could mediate the passive transport of a solute. The transporter shown can exist in two conformational states: in state A, the binding sites for solute are exposed on the outside of the lipid bilayer; in state B, the same sites are exposed on the other side of the bilayer. The transition between the two states can occur randomly. It is completely reversible and does not depend on whether the solute binding site is occupied. Therefore, if the solute concentration is higher on the outside of the bilayer, more solute binds to the carrier protein in the A conformation than in the B conformation, and there is a net transport of solute down its concentration gradient (or, if the solute is an ion, down its electrochemical gradient).

El transporte activo es efectuado por proteínas transportadoras acopladas a una fuente de energía

Three ways of driving active transport. The actively transported molecule is shown in *yellow,* and the energy source is shown in *red.*

Three types of carrier-mediated transport. This schematic diagram shows carrier proteins functioning as uniporters, symporters, and antiporters.

One way in which a glucose carrier can be driven by a Na$^+$ gradient. In the model the transporter oscillates between two alternate states, A and B. In the A state, the protein is open to the extracellular space; in the B state, it is open to the cytosol. Binding of Na$^+$ and glucose is cooperative that is, the binding of either ligand induces a conformational change that greatly increases the protein's affinity for the other ligand. Since the Na$^+$ concentration is much higher in the extracellular space than in the cytosol, glucose is more likely to bind to the carrier in the A state. Therefore, both Na$^+$ and glucose enter the cell (via an A →B transition) much more often than they leave it (via a B → A transition). The overall result is the net transport of both Na$^+$ and glucose into the cell. Note that because the binding is cooperative, if one of the two solutes is missing, the other fails to bind to the carrier. Thus, the carrier undergoes a conformational switch between the two states only if both solutes or neither are bound.

Transcellular transport. The transcellular transport of glucose across an intestinal epithelial cell depends on the nonuniform distribution of transport proteins in the cell's plasma membrane. The process shown here results in the transport of glucose from the intestinal lumen to the extracellular fluid (from where it passes into the blood). Glucose is pumped into the cell through the apical domain of the membrane by a Na$^+$ -powered glucose symport. Glucose passes out of the cell (down its concentration gradient) by passive transport mediated by a different glucose carrier protein in the basal and lateral membrane domains. The Na$^+$ gradient driving the glucose symport is maintained by a Na$^+$ pump in the basal and lateral plasma membrane domains, which keeps the internal concentration of Na$^+$ low. Adjacent cells are connected by impermeable tight junctions, which have a dual function in the transport process illustrated: they prevent solutes from crossing the epithelium between cells, allowing a concentration gradient of glucose to be maintained across the cell sheet, and they also serve as diffusion barriers within the plasma membrane, which help confine the various carrier proteins to their respective membrane domains.

Three types of ATP-driven pumps. The different molecular designs are shown. Like an enzyme, pumps can work in reverse; when the electrochemical gradients of the solutes are reversed and the ATP/ADP ratio is low, they can synthezise ATP from ADP and Pi, as shown for the F-type ATPase, which normally works in this mode

The Na⁺-K⁺pump. This transporter protein actively pumps Na⁺ out of and K⁺ into a cell against their electrochemical gradients. For every molecule of ATP hydrolyzed inside the cell, three Na⁺ are pumped out and two K⁺ are pumped in. The specific inhibitor ouabain and K⁺ compete for the same site on the extracellular side of the pump.

A model of the pumping cycle of the Na⁺ -K⁺ pump

Figure 11-16. Response of a human red blood cell to changes in osmolarity of the extracellular fluid. The cell swells or shrinks as water moves into or out of the cell down its concentration gradient.

A model of how the sarcoplasmic reticulum Ca²⁺ pump moves Ca²⁺.

A model of how the sarcoplasmic reticulim Ca²⁺ moves Ca²⁺. (A) The structures of the unphospohorylated, Ca²⁺-bound state (*left*) and the phospohorylated, Ca²⁺-free state (*right*). (B) The model shows haw ATP binnding and hydrolysis cause drastic conformational changes, bringing the nucleotide binding and phosphorylation domains in close proximity. This change is thought to cause a 90° rotation of the activator domain, which leads to a rearrangement of the transmembrane helices. The rearrangement of helices disrupts the Ca²⁺ -binding cavity and releases the Ca²⁺ into the lumen of the sarcoplasmic reticulum

A typical ABC transporter. (A) A topology diagram. (B) A hypothetical arrangement of the polypeptide chain in the membrane. The transporter consists of four domains: two highly hydrophobic domains, each with six putative membrane-spanning segments that somehow form the translocation pathway, and two ATP-binding catalytic domains (or cassettes). In some cases the two halves of the transporter are formed by a single polypeptide (as shown), whereas in other cases they are formed by two or more separate polypeptides that assemble into a similar structure.

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Los canales iónicos son selectivos y alternan entre el estado abierto y el estado cerrado

A typical ion channel, which fluctuates between closed and open conformations. The channel protein shown here in cross section forms a hydrophilic pore across the lipid bilayer only in the "open" conformational state. Polar groups are thought to line the wall of the pore, while hydrophobic amino acid side chains interact with the lipid bilayer (not shown). The pore narrows to atomic dimensions in one region (the selectivity filter), where the ion selectivity of the channel is largely determined.
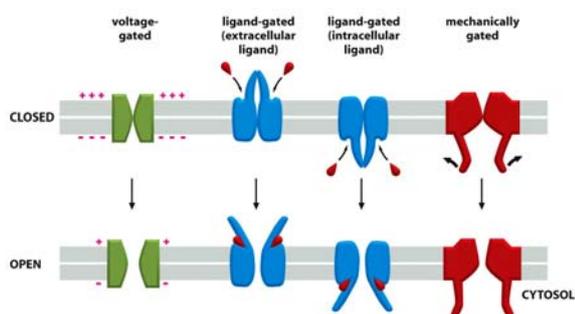
The gating of ion channels. This drawing shows different kinds of stimuli that open ion channels. Mechanically gated channels often have cytoplasmic extensions that link the channel to the cytoskeleton (not shown).
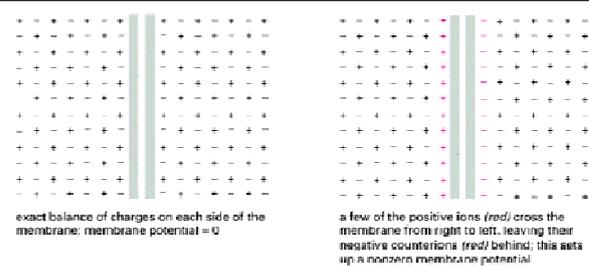
The ionic basis of a membrane potential. A small flow of ions carries sufficient charge to cause a large change in the membrane potential. The ions that give rise to the membrane potential lie in a thin (< 1 nm) surface layer close to the membrane, held there by their electrical attraction to their oppositely charged counterparts (counterions) on the other side of the membrane. For a typical cell, 1 microcoulomb of charge (6×10^{12} monovalent ions) per square centimeter of membrane, transferred from one side of the membrane to the other, changes the membrane potential by roughly 1 V. This means, for example, that in a spherical cell of diameter 10 μm, the number of K^+ ions that have to flow out to alter the membrane potential by 100 mV is only about 1/100,000 of the total number of K^+ ions in the cytosol.

The structure of a bacterial K⁺ channel. (A) Only two of the four identical subunits are shown. From the cytosolic side, the pore opens up into a vestibule in the middle of the membrane. The vestibule facilitates transport by allowing the K⁺ ions to remain hydrated even though they are halfway across the membrane. The narrow selectivity filter links the vestibule to the outside of the cell. Carbonyl oxygens line the walls of the selectivity filter and form transient binding sites for dehydrated K⁺ ions. Two K⁺ ions occupy sites in the selectivity filter, while a third K⁺ ion is located in the center of the vestibule, where it is stabilized by electrical interactions with the more negatively charged ends of the pore helices. The ends of the four pore helices point precisely toward the center of the vestibule, thereby guiding K⁺ ions into the selectivity filter. (B) Because of the polarity of the hydrogen bonds (in *red*) that link adjacent turns of an α helix, every α helix has an electric dipole along its axis, with a more negatively charged C-terminal end (δ^-) and a more positively charged N-terminal end (δ^+). (A, Adapted from D.A. Doyle et al., *Science* 280:69–77, 1998.)

	Na⁺	K⁺
Número atómico	11	19
Radio covalente (pm)	154	196
Radio iónico (pm)	95	133
Radio atómico (pm)	190	235
Configuración	$3s^1$	$4s^1$

K⁺ specificity of the selectivity filter in a K⁺ channel. The drawing shows K⁺ and Na⁺ ions (A) in the vestibule and (B) in the selectivity filter of the pore, viewed in cross section. In the vestibule, the ions are hydrated. In the selectivity filter, the carbonyl oxygens are placed precisely to accommodate a dehydrated K⁺ ion. The dehydration of the K⁺ ion requires energy, which is precisely balanced by the energy regained by the interaction of the ion with the carbonyl oxygens that serve as surrogate water molecules. Because the Na⁺ ion is too small to interact with the oxygens, it could enter the selectivity filter only at a great energetic expense. The filter therefore selects K⁺ ions with high specificity. (Adapted from D.A. Doyle et al., *Science* 280:69–77, 1998.)

Table 11-2. Some Ion Channel Families

FAMILY*	REPRESENTATIVE SUBFAMILIES	
Voltage-gated cation channels	voltage-gated Na⁺ channels voltage-gated K⁺ channels (including delayed and early) voltage-gated Ca²⁺ channels	
Transmitter-gated ion channels	acetylcholine-gated cation channels glutamate-gated Ca²⁺ channels serotonin-gated cation channels	} excitatory
	GABA-gated Cl⁻ channels glycine gated Cl⁻ channels	} inhibitory

*The members of a family are similar in amino acid sequence and are therefore thought to have derived from a common ancestor; within subfamilies, the resemblances are usually even closer.
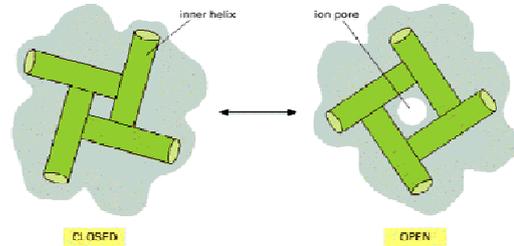
A model for the gating of a bacterial K⁺ channel. The channel is viewed in cross section. To adopt the closed conformation, the four inner transmembrane helices that line the pore on the cytosolic side of the selectivity filter rearrange to close the cytosolic entrance to the channel. (Adapted from E. Perozo et al., *Science* 285:73–78, 1999.)

Las causas de la osmolaridad intracelular
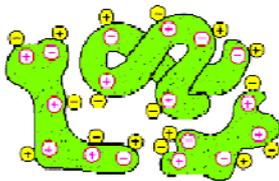
La contribución de las **macromoléculas** a la osmolaridad intracelular es más bien pequeña, puesto que a pesar de ser grandes su numero es pequeño cuando se lo compara con el número de moléculas de pequeño tamaño que se encuentran al interior de una célula. Sin embargo, la mayoría de las macromoléculas presentan carga y como consecuencia atraen iones de carga opuesta. Debido a su elevado número, estos **contraiones** representan una contribución importante a la osmolaridad intracelular.

Las causas de la osmolaridad intracelular
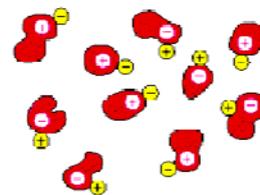
Como resultado de los procesos metabólicos y del transporte activo, las concentraciones intracelulares de **moléculas orgánicas pequeñas** para las cuales la membrana plasmática es impermeable(azúcares,aminoácidos, nucleótidos) son altas. Debido a que la mayoría de estos metabolitos poeen carga, atraen contariones al igual que las macromoléculas. De este modo, tanto **los metabolitos pequeños** como sus **contraiones** contribuyen a la osmolaridad intracelular.

Las causas de la osmolaridad intracelular
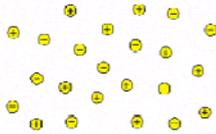
Generalmente, la osmolaridad del medio extracelular se debe principalmente a iones inorgánicos pequeños. Estos iones se filtran ("gotean") lentamente a través de la membrana plasmática hacia el interior de la célula. Si estos iones fueran devueltos al exterior, y si no hubiera otras moléculas intrecelulares que interactuaran con ellos de modo que esto afectara su distribución, dichos iones alcanzarían eventualmente un equilibrio, de modo que la concentración intracelular sería igual a la concentración extracelular. No obstante, la presencia de metabolitos y macromoléculas cargadas al interior de la célula, que interactuan con estos iones, origina el **efecto Donnan**: esto significa que la concentración total de iones inorgánicos (y por lo tanto su contribución a la osmolaridad) será mayor dentro que fuera de la célula en el equilibrio.

¿Cuál es el problema?
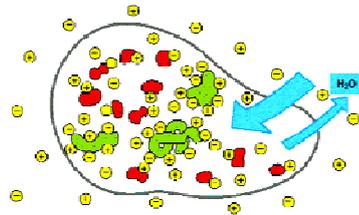
A causa de los factores mencionados, una célula que no dispone de un mecanismo para controlar su osmolaridad tendrá una mayor concentración de solutos al interior que al exterior. Como consecuencia, la concentración del agua será mayor afuera de la célula que dentro de ella. Esta diferencia de concentración de agua a través de la membrana plasmática hará que el agua ingrese continuamente a la célula por **osmosis**, causando finalmente su ruptura

Las soluciones
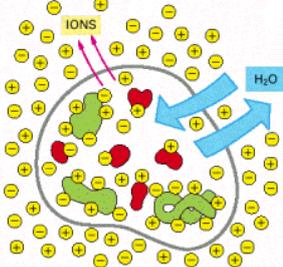
Las células animales y las bacterias controlan la osmolaridad intracelular bombeando activamente hacia el medio extracelular iones inorgánicos, como por ejemplo Na^+, de modo que su citoplasma contiene una concentración total de iones menor que la del medio extracelular, compensado de este modo su exceso de solutos orgánicos.

Las soluciones
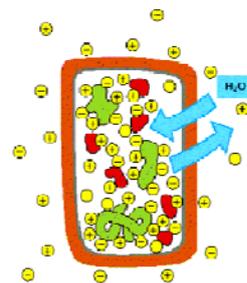
La pared celular de celulosa que poseen las células vegetales evita que estas se hinchen, de modo que pueden tolerar una diferencia osmótica a través de su membrana plasmática; la presión de turgencia interna resultante, en el equilibrio, fuerza la salida de agua de modo que la cantidad de agua que sale es igual a la cantidad de agua que entra.

Las soluciones

Muchos protozoos evitan el hincharse con agua, a pesar de las diferencias osmóticas a través de la membrana plasmática, eliminando periodicamente agua mediante el uso de una vacuola contráctil asociada a elementos del citoesqueleto.

La ecuación de Nernst y el flujo de iones

El flujo de un ión cualesquiera a través de una membrana es impulsado por la **gradiente electroquímica** para ese ión. Esta gradiente representa la combinación de dos componentes: la gradiente de voltaje y la gradiente de concentración a través de la membrana. Cuando estos dos componentes se contrabalancean la gradiente electroquímica para ese ión es cero y por tanto no hay flujo neto del ión a través del canal. La gradiente de voltaje (potencial de membrana) a la cual se alcanza este equilibrio se denomina **potencial de equilibrio** para ese ión. Este valor puede calcularse a partir de la **ecuación de Nernst**. La ecuación de Nernst tiene la forma:

$$V = \frac{RT}{zF} \ln \frac{C_o}{C_i}$$

V: potencial de equilibrio en volts (potencial interno menos potencial externo)
C_o y C_i: concentraciones externa e interna, respectivamente, del ión
R: Constante de los gases (1.98 cal*mol^{-1}*K^{-1})
T: temperatura absoluta (K)
F: constante de Faraday (23062 cal*Volt^{-1}*mol^{-1})
z: carga del ión

Como construir la ecuación de Nernst

Una molécula en solución (un soluto) tiende a moverse desde una región donde su concentración es alta hacia regiones donde su concentración es baja (difusión), lo que resulta en una distribución en equilibrio de ese soluto. Como resultado, el movimiento a favor de la gradiente de concentración se acompaña por un cambio de energía libre favorable ($\Delta G < 0$) en tanto que el movimiento en contra de la gradiente de concentración se acompaña de un cambio de energía libre desfavorable ($\Delta G > 0$). El cambio de energía libre por mol de soluto que se mueve a través de la membrana (ΔG_{conc}) es igual a $-RT \ln C_o/C_i$. Si el soluto es un ion que se mueve a través de la membrana hacia el interior de la célula cuyo interior se encuentra a un voltaje V relativo al exterior, causará un cambio de energía libre adicional (por mol de soluto transportado) de $\Delta G_{volt} = zFV$. En este punto, donde las gradientes de voltaje y de concentración se balancean exactamente $\Delta G_{conc} + \Delta G_{volt} = 0$ y la distribución del ion está en equilibrio a través de la membrana. Así:

$$zF\,V - RT \ln \frac{C_o}{C_i} = 0$$

y por tanto

$$V = \frac{RT}{zF} \ln \frac{C_o}{C_i} \quad = \quad V = 2.3 \frac{RT}{zF} \log \frac{C_o}{C_i}$$

Para un ion monovalente el valor de $2.3\,RT/F$ es de 57.9 mV a 20 ºC y de 61.5 mV a 37 ºC. De este modo, para un ion monovalente, a 37 ºC, V= 61.5 mV para C_o/C_i =10 en tanto que V=0 para C_o/C_i=1.

En el caso del K$^+$, a 37 ºC, el potencial de equilibrio (V_K) es 61.5*log (($K^+)_o/(K^+)_i$) mvolts (-89 mvolts para una célula típica en que $[K]_o$ = 5 mM y $[K_i]$ =140 mM). A este potencial no hay flujo neto de K$^+$ a través de la membrana. Del mismo modo, cuando el potencial de membrana tiene un valor de 61.5*log (($Na^+)_o/(Na^+)_i$), el potencial de equilibrio de sodio, (V_{Na}), no hay flujo neto de Na$^+$.

Para cualquier valor de potencial de membrana V_M, la fuerza neta que ayuda a impulsar un ión fuera de la célula es proporcional a la diferencia entre V_M y el potencial de equilibrio para ese ión. Para el K$^+$ el valor resultan ser V_M - V_K en tanto que para el Na$^+$ es V_M - V_{Na}

El número de iones que se necesitan para formar una capa de carga adyacente a la membrana es pequeño cundo se lo compara con el número total de iones dentro de una célula. El movimiento de, por ejemplo, 6000 iones Na$^+$ a través de 1 μm^2 de membrana llevará suficiente carga para cambiar el potencial de membrana en alrededor de 100 mV. Debido a que existen típicamente unos $3*10^7$ iones Na$^+$ en una célula (1 μm^3 de volumen de citoplasma) un movimiento de iones de esa magnitud tendrá un efecto despreciable sobre la gradiente de concentración a través de la membrana.