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Ion Channels

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Advanced Article

Article Contents

Ion channels catalyze the transmembrane movement of small inorganic ions across biological membranes. They do so by forming continuous, hydrophilic pores through which ions can cross the barrier imposed by the lipid bilayer hydrophobic core. Ion channels serve many functions: they underlie the changes in membrane potential that control many cell functions, including the propagated electrical signaling (the action potentials) in electrically excitable cells; they allow for the bulk movement of ions across cell membranes. In this article we summarize key features of ion channels, with special emphasis on the channels in the plasma membrane—their structure and catalytic power, the generation of membrane potential changes, the regulation (or gating) that underlies normal channel function, and how channel function can be modulated by small molecules.

Introduction

Bilayer-spanning ion channels are ubiquitous constituents of prokaryotic and eukaryotic plasma (and organellar) membranes (1); channel-encoding genes are found even in viral genomes (2, 3). Ion channels serve many biological functions by being conduits for rapid, regulated ion movement across cellular membranes. Notably, they allow for the changes in membrane potential that underlie the function of the nervous system (4) and other excitable tissues.

Ion conducting channels catalyze transmembrane ion movement by forming continuous paths through which ions can cross the barrier imposed by the membranes' lipid bilayer moieties (1, 5, 6). In biological membranes, integral membrane proteins provide the structural basis for ion channels by forming the walls of water-filled, high-dielectric pores that allow for electrodiffusive ion movement from one aqueous phase to the other. Ion channels thus provide an alternate permeation (or reaction) path for transmembrane ion movement, in the sense that the permeating ions do not cross the membrane by moving through the bilayer hydrophobic core per se (**Fig. 1**).

Ion channels are not just water-filled pores

Most channels are not just "simple" water-filled pores; they possess substrate specificity (ion selectivity) and so can discriminate between chemically closely related ion species such as K^+ and Na^+ (1). That is, ion channels are highly specific protein catalysts or enzymes (7, 8). Ion channels constitute a special class of enzymes, however, because no covalent bonds are made or broken during the ions' passage through a channel pore.

Although channel pores generally are water-filled, some pores are so narrow that the water chain is interrupted by the permeating ions, as shown for the selectivity filter in potassium channels (9), in which the water molecules are separated by K^+. It also has been proposed that H^+ movement through voltage-dependent proton channels proceeds along a hydrogen-bonded network that may not contain a continuous chain of water molecules (10).

Ion channels are efficient catalysts of transmembrane ion movement

Similar to other enzymes, the important determinants of ion channel function are: the channels' substrate specificity (ion selectivity), their turnover number and catalytic power, and their regulation. Because no covalent bonds are formed or broken when ions pass through a channel, the energy barrier for channel-catalyzed ion movement may be quite low. A distinguishing feature of ion channels, therefore, is their turnover number, which can be $>10^8$ s^{-1} (11)—as compared with $<10^6$ s^{-1} for most regular enzymes (see Ref. 1, Table 11.2A)—and a turnover number greater than 10^5 s^{-1} is commonly considered to be a defining feature of ion channels. Though there is no lower bound on the ion flux through membrane-spanning channels is

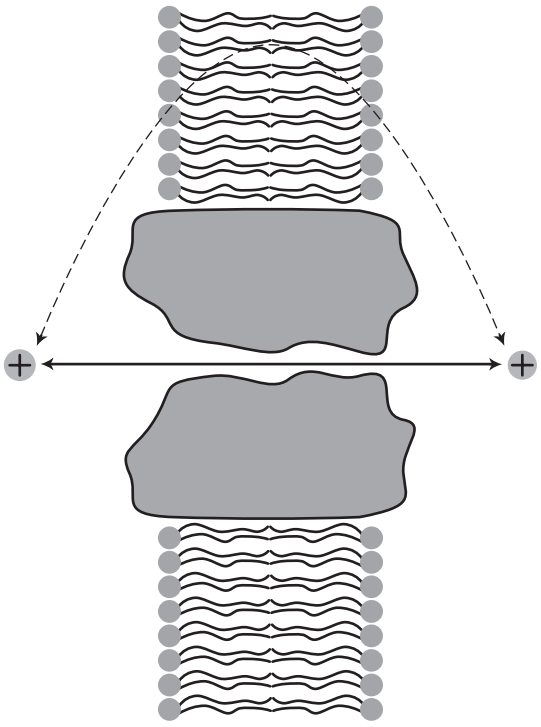

Figure 1 Channel-mediated ion movement. The channel protein forms the walls of an aqueous pore through which ions, water, and other small solutes can move across the membrane (indicated by the solid line). Ions also can traverse the membrane by moving through the bilayer itself (indicated by the interrupted curve). Because the solubility of small inorganic ions in the membrane interior is very low, this uncatalyzed ion movement through the lipid bilayer core usually can be ignored.

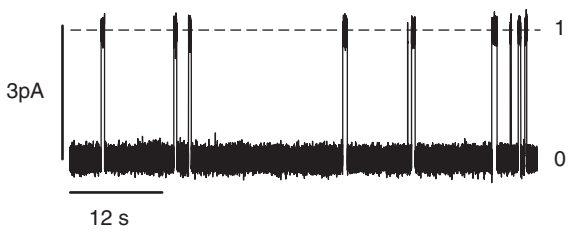

Figure 2 Single-channel current trace recorded in planar lipid bilayers doped with gramicidin A (gA). The gA channels do not open and close but form and disappear. The numbers to the right in the figure indicate the number of conducting channels; the stippled line indicates the current level for the conducting channel. Experimental conditions as in 12: 1.0 M NaCl, 200 mV applied potential, and dioleoylphosphatidylcholine/*n*-decane bilayer.

found, the typically very high turnover allows for electrophysiological measurements of ion movement (the single-channel current) through individual channels (**Fig. 2**).

Indeed, the first single-molecule measurements were single-channel recordings (13, 14), which provide the ability to resolve single-channel current transitions (single protein conformational transitions) at the μs timescale (15, 16). The high temporal resolution provided by electrophysiological measurements makes ion channels important not only because of their intrinsic physiological functions but also because they can be used as tools to study general features of (membrane) protein function.

Ion movement through ion channels occurs as an electrodiffusive barrier crossing, in which the ion movement is largely uncoupled from protein conformational transitions. To appreciate ion channels' catalytic power, it is instructive to compare the measured conductances with predictions based on a simple water-filled pores with solvent properties similar to bulk water, while neglecting the diffusional access resistance to the channel entrance (17, 18). In this case, the predicted single-channel conductance for monovalent ions, g_{pred}, becomes (Ref. 19, p. 51):

$$g_{\text{pred}} \approx \lambda^{\circ} \cdot \mathrm{C} \cdot \frac{\pi \cdot (r_p - r_i)^2}{l_p} \qquad (1)$$

where λ° is the limiting molar conductivity (a measure of the ion mobility in bulk aqueous solutions, units S·cm^2/mole), C the permeant ion concentration in the bulk aqueous phase, r_p the pore radius, r_i the ionic radius, and l_p the pore length. [The appropriate radius in Eq 1 is the difference between r_p and r_i because the ion centers are constrained to move within a narrow cylinder of radius $r_p - r_i$ (20).] For Na^+ permeation through the cation selective gA channels [$\lambda^{\circ}_{\text{Na}} = 50.1$ S·cm^2/mole (21), $r_{\text{Na}} \approx 0.95$ Å (22), $r_p = 2$ Å, and $l_p = 25$ Å (23)], g_{pred} is predicted to be about ≈ 70 pS in 1.0 M NaCl and 7 pS in 0.1 M NaCl—similar to the experimental values of 12.5 pS (in 1.0 M NaCl) and 5.3 pS (in 0.1 M NaCl) (23). This similarity does not mean that ion channels are just aqueous pores. Ions may bind with high affinity into the pore of ion channels, such that the ion mole-fraction in the pore may be several orders of magnitude higher than in the aqueous solution (see next section).

Catalytic rate enhancement

It is possible to extend this argument by estimating the catalytic rate enhancement provided by an ion channel (19). By analogy with conventional enzymes (24), the catalytic rate enhancement can be defined as the rate of channel-mediated ion movement (k_{cat}) relative to the rate of noncatalyzed movement through the bilayer (k_{non}). To a first approximation, $k_{\text{cat}}/k_{\text{non}}$ can be equated with $K_{w\to p}/K_{w\to m}$, where $K_{w\to p}$ and $K_{w\to m}$ denote the ion partition coefficients from bulk water into the pore and the bilayer hydrophobic core, respectively. $K_{w\to p}$ can be estimated from apparent dissociation constants for the permeant ions, which are in the μM range for calcium (25) and potassium (26) channels. That is, $K_{w\to p}$ can be approximated as $n_{\text{ion}}^{\text{pore}}/n_{\text{ion}}^{\text{bulk}}$, where $n_{\text{ion}}^{\text{pore}}$ and $n_{\text{ion}}^{\text{bulk}}$ denote the ion mole-fractions in the pore and the bulk solution, which can be as high as 10^6. $K_{w\to m}$ can be estimated from the "leak" conductance ($G_0 \approx 10^{-9}$ S/cm^2) of unmodified bilayers in 1.0 M salt (27) using the relation (7, 28):

$$G_0 = N_A \cdot \frac{(z \cdot e)^2}{k_B T} \cdot \frac{D_m}{d_0} \cdot K_{\text{w}\to\text{m}} \cdot C \qquad (2)$$

where N_A is Avogadro's number, k_B Boltzmann's constant, T the temperature in Kelvin, z the ion's valence, e the elementary charge, and D_m the ion's diffusion coefficient in the bilayer hydrophobic core [about 10^{-5} cm^2·s^{-1} (29)]. Based on Eq. 2, $K_{w\to m}$ is predicted to be 10^{-14} (or less), such that the catalytic rate enhancement may be as high as 10^{20}—higher than the rate enhancements observed for conventional enzymes (24).

Channels and Diseases

Because ion channels are such effective catalysts for transmembrane ion movement, disruptions in channel function can lead to disease, and an increasing number of human diseases have been shown to be caused by abnormal channel function caused by mutations in channel genes [(30, 31); see also Ion Channels in Medicine]. These so-called channelopathies can develop from defective channel synthesis that is caused by missense mutations or splice defects (31); inappropriate trafficking of the mutant channels to their target membrane [as is the case for the cystic fibrosis transmembrane conductance regulator or CFTR channel (32)], or channel retrieval [as is the case for the epithelial sodium channel, ENaC, in Liddle's syndrome (33)]; also, they can develop from altered gating, which may increase or decrease the number of conducting channels (34); altered ion selectivity, which may disrupt the cellular electrolyte and volume balance (35); or the appearance of new channel functions [such as novel pathways for ion movement (36), which again may compromise the cellular electrolyte and volume balance (37)].

Diversity of Ion Channels

Ion channels can be classified based on their structure and function. Structurally, ion channels usually are hetero- or homo-oligomers of pore-forming subunits, sometimes in association with accessory/modulatory subunits. Channels in plasma membranes have predominantly α-helical bilayer-spanning (or transmembrane, TM) domains, whereas channels in bacterial outer membranes (and the outer membrane of mitochondria) have a predominantly β-barrel structure (**Fig. 3**). Although the focus usually is on the TM domains, for many channels, the majority of the molecular mass is outside the membrane, illustrated in **Fig. 3** for a Kir3.1 chimera and nAChR.

Functionally, ion channels are distinguished by their permeability and gating properties and by their cellular localization. Most channels are valence selective, and many channels exhibit exquisite selectivity among ions of the same valence [as indicated by their names—calcium channels, chloride channels, potassium channels, and sodium channels, for example, (1)]. The major exception are the large-diameter channels, such as the porins in the outer membranes of Gram-negative bacteria and the connexins (or gap junctions) that couple adjacent cells together—both electrically and by mediating cell-to-cell movement of low-molecular weight signaling molecules (42). These channels allow for the relatively nonselective transmembrane movement of both anion and cations (43), yet such channels may exhibit selectivity among (larger) organic solutes (43–45). Indeed, although channels with large-diameter pores allow for the passage of large solutes, they do not necessarily have high single-channel conductances; no clear relation is found between a channel's pore dimensions and its conductance (46).

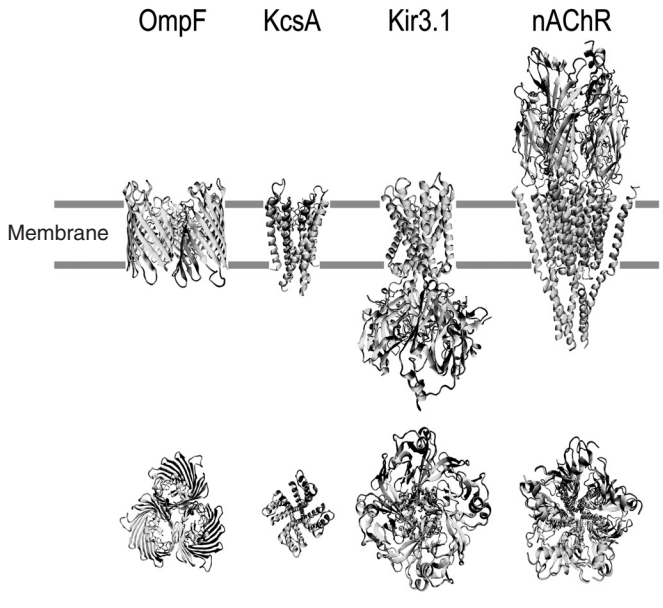

Figure 3 Structural representation of different transmembrane ion channels (from left to right): the bacterial outer membrane protein OmpF [PDBid: 1OPF (38)]; the bacterial potassium channel KcsA [PDBid: 1K4C (39)]; a chimera between the mammalian inward rectifier potassium channel Kir3.1 and the bacterial inward rectifier KirBAC1.3 [PDBid: 2QKS (40); in this structure a large part of the transmembrane pore domain of Kir3.1 has been replaced by the corresponding fragment of the pore domain of the prokaryotic KirBac1.3]; and the mammalian nicotinic acetylcholine-gated channel nAChR [PDBid: 2BG9 (41)]. α-helices are indicated as coiled ribbons, β-sheets as straight ribbons, and the loops as lines. Top row: Side views of the channels relative to the outer membrane (OmpF, with the periplasmic surface down) or the plasma membrane (KcsA, Kir3.1, and nAChR, with the intracellular surface down). Bottom row: the channels viewed along the pore: OmpF is viewed from the periplasmic solution; KcsA and nAChR are viewed from the extracellular solution; and Kir3.1 is viewed from the intracellular solution. The biological unit of the OmpF consists of three functional channels, and each pore is lined by a single subunit. The KcsA and Kir3.1 channels are formed by four subunits that line a single pore; the nAChR channel is formed by five subunits that line a single pore.

Ion channel function may be altered by changes in the surface density of channels in the membrane (see Dynamics of Cell Membranes) as well as by rapid transitions between nonconducting and conducting channel states—a process denoted as channel gating. The two classic examples of channel gating are: first, the *voltage-dependent gating* (described in ~~the section titled~~ "Voltage-Dependent Gating") of sodium and potassium channels that underlie the action potentials in nerve and muscle cells, in which the channel state is controlled by the transmembrane potential, and second, the *ligand-dependent gating* (described in ~~the section titled~~ "Ligand Activation") of the channels involved in synaptic (chemical) transmission of electrical signals from cell to cell, such as the nicotinic acetylcholine receptors in skeletal muscle, in which the channel state is controlled by ligand (acetylcholine) binding (1). The principles underlying channel gating will be discussed in ~~the section titled~~ "Channel Gating."

Classification of ion channels

Table 1 lists ion channels according to their gene superfamilies, number of TM (α-helical) segments, and function. The emphasis is on plasma membrane channels because most is known about them.

Pore-loop channels

The largest family of plasma membrane ion channels is the family of pore-loop channels, listed in **Table 1a**. The defining feature of these channels is their central core, which forms the ion permeable pore with the pore lining being formed by two TM α-helices plus the intervening sequence that loops into the membrane to form the selectivity filter—the pore region that determines which ions can permeate the channel. The archetypical example of this so-called 2TM,1P (P for pore) motif is found in the KcsA channel (**Fig. 3**), the first

Table 1a Ion channel classes: plasma membrane pore-loop and cys-loop channels

Channel class	Organization	Type	Properties	Reference
Pore-loop channels	2TM, 1P (tetramers)	KcsA	K^+ selective (proton-gated)	(9) (47, 48)
		Kir	K^+ selective (inward-rectifying)	(49) (50)
	4TM,2P (dimers)	K_{2P}	K^+ selective (nonrectifying; stretch- and amphiphile-gated)	(51) (52)
		K_V	K^+ selective (voltage-dependent)	(53) (54)
		M channels	K^+ selective (voltage-dependent; G protein-inhibited)	(55)
		hERG	K^+ selective (slow voltage-dependent)	(56)
	6TM,1P (tetramers)	K_{Ca} (SK)	K^+ selective (Ca^{2+}/calmodulin-activated	(57) (58)
		CNG	Cation selective (cyclic nucleotide-dependent)	(59)
		HCN	Cation selective (voltage- and cyclic nucleotide-dependent)	(60)
		TRP	Cation selective (sensory, mechano-, and amphiphile-activated)	(61) (62)
	7TM,1P (tetramers)	K_{Ca} (BK)	K^+ selective (Ca^{2+}- and voltage-activated)	(57) (63)
	24TM,4P (monomers)	Na_V	Na^+ selective (voltage-activated)	(64) (65)
		Ca_V	Ca^{2+} selective (voltage-activated)	(66) (67)
	3TM,1P (monomers)	GluR	Cation selective (glutamate-activated)	(68) (69)
Cys-loop channels	4TM (pentamers)	AChR, $5HT_3R$, $GABA_AR$, GlyR	Cation selective (acetylcholine- or serotonin-activated) Anion selective (γ-aminobutyric acid- or glycine-activated)	(41) (70) (71) (72)

ion-selective plasma membrane channel whose high-resolution structure was determined (9). The channel is a tetramer of four pore-forming 2TM,1P subunits, in which the selectivity filter is lined by four extended peptide strands (one from each subunit) in a loop between the TM helices. This bilayer-spanning motif is found not only in KcsA but also in the Kir family of inward rectifier potassium channels (50); the latter class of channels also has a large cytoplasmic domain (cf. **Fig. 3**).

The basic 2TM,1P motif has evolved in three different directions (**Fig. 4**). The first direction evolved by a process of internal gene duplication that led to the so-called 2-pore (K_{2P}) channels with a 4TM,2P organization. The second direction evolved by a more complex process that involved an inversion of the transmembrane topology, the accretion of an additional TM segment, plus an extracellular domain with sequence similarity to bacterial periplasmic amino acid binding proteins (96), which led to the glutamate receptor family of ligand-activated channels with a 3TM,1P organization. And the third direction evolved by acquiring a 4TM voltage-sensing domain [that by itself can form a voltage-dependent proton channel (H_V) (97)] that led to the 6TM,1P group of channels, which are largely potassium selective and include the voltage-dependent potassium channels (K_V). This 6TM,1P motif has evolved into the 7TM,1P family of high-conductance Ca^{2+}-activated potassium channels (K_{Ca} or BK), which also have large C-terminal Ca^{2+} binding domains. Finally, the 6TM,1P motif also evolved by internal gene duplication to form the so-called two-pore channels that have two domains and the voltage-dependent sodium (Na_V) and calcium (Ca_V) channels that have four domains. In both cases, each domain corresponds to a duplicated 6TM,1P pore-loop motif. [The emphasis in **Table 1** and **Fig. 4** is on the pore-forming subunits, called α subunits; functional channels usually are coassemblies of four pore-forming subunits or domains plus additional cytoplasmic or transmembrane regulatory subunits (1).]

Cys-loop channels

Another major family of plasma membrane ion channels is the family of ligand-gated Cys-loop receptors, also listed in **Table 1a**. These channels can be subdivided based on their activating ligand: acetylcholine (ACh) for the nicotinic Ach receptor (nAChR, **Fig. 3**); serotonin (5-HT) for the 5-HT_3R; glycine (Gly) for the GlyR; and γ-aminobutyric acid (GABA) for the $GABA_A$R. ACh, 5-HT, and GABA also bind to other receptors, for example, muscarinic AChR and $GABA_B$R, which are G-protein coupled receptors (see Large G-Proteins and Drug Design Strategies for Targeting G-Protein-Coupled Receptors). These channels also can be categorized based on their ion selectivity and function: nAChR and 5-HT_3R are cation selective channels involved in excitatory synaptic transmission; $GABA_A$R and GlyR are anion selective channels involved in inhibitory synaptic transmission. [In addition to the listed channels, a H^+-gated Cys-loop channel has been reported (98).]

Other channel types

The remaining channel types listed in **Table 1b** are more difficult to categorize, and many of them have no obvious evolutionary relationship.

The major exception is the family of epithelial sodium channels (ENaC, for epithelial Na channel), the mechanosensitive channels (Deg, for degeneration because Deg activation triggers cell death) first identified in *Caenorhabditis elegans*, and the H^+-gated sodium channels (ASIC, for acid sensitive ion channel) found in various cell types. These channels are related also to a family of invertebrate peptide-gated channels [the FMRFamide-gated channels (99)].

There also may be an evolutionary relationship between the intracellular Ca^{2+} release channels, the ryanodine receptors (RyR) that mediate the release of Ca^{2+} from the sarcoplasmic reticulum, and the IP_3 receptors (IP_3R) that mediate the release of Ca^{2+} from many intracellular organelles.

In the family of ClC proteins, some members are expressed in the plasma membrane where they form anion selective channels. These channels occur as dimers, with each subunit forming a transmembrane pore. Other members of this family are expressed in organellar membranes, where many of them form H^+/Cl^- countertransporters (100), as first demonstrated for an bacterial plasma membrane ClC protein (101)—thereby validating a conjecture by Peter Läuger (102) that there would be a continuum of molecular mechanisms catalyzing transmembrane solute movement, ranging from conformational transporters to transmembrane channels.

The porins in the outer bacterial and mitochondrial membranes serve as conduits to the periplasmic space. Some porins occur as oligomers, each forming a transmembrane pore (**Fig. 3**). Many porins and the voltage-dependent anion-selective channel (VDAC) appear to catalyze the relatively nonspecific transfer of solutes below a certain size; other porins are involved in more specific solute transfer (103). The H^+ permeable M2 channel, which is encoded by the influenza virus, is important for viral replication and is the target for the antiviral drug amantadine (95).

Ion Permeation and Membrane Potentials

Most cell membranes are endowed with different types of ion channels (**Fig. 5**) that differ in their ion selectivity and gating properties (cf. **Table 1**).

Because the extracellular and intracellular ion concentrations differ (**Table 2**), the membrane potential ($V_m = V_i - V_e$, where V_i and V_e denote the electrical potential of the intracellular and extracellular compartment, respectively) will vary as a function of the number and type of conducting channels in the membrane (see ~~the section titled~~ "Membrane Potentials and Single-Channel Currents).

Although channel-catalyzed ion movement occurs as an electrodiffusive barrier crossing (7, 105–110), the permeating ions usually are not distributed uniformly along the pore; they tend to be localized in discrete regions, or energy minima (e.g., see Ref. 9), such that the ion movement can be approximated as a series of discrete transitions:entry, translocation through the pore, and exit (**Fig. 6**).

[Although the kinetics of channel-mediated ion movement may be described using discrete-state kinetics, the individual

Table 1b Ion channel classes: channels other than pore-loop and cys-loop channels

Localization	Organization	Type	Properties	Reference
Plasma membrane	4TM	CRAC	Ca^{2+} selective (Ca^{2+} store depletion-activated)	(73)
	4TM	H_v	H^+ selective (voltage-dependent)	(74) (75)
		ENaC	Na^+ selective	(76) (77)
	2TM (trimers)	Deg	Na^+ selective (mechanosensitive)	(78)
		ASIC	Na^+ selective (H^+-activated)	(79) (80)
	2TM (trimers)	P_2X	Ca^{2+} (cation) selective (ATP-dependent)	(81) (82)
	10TM (dimers)	ClC	Cl^- (anion) selective (some are Cl^-/H^+ antiporters) (Cl^-- and voltage-dependent)	(83) (84)
	12TM (monomers)	CFTR	Cl^- (anion) selective (pKA-dependent phosphorylation)	(85)
	4TM (hexamers)	Connexins	Selectivity among cytoplasmic signaling molecules (H^+-gated)	(42) (43)
		Pannexins		(86)
	4TM		Selectivity among cytoplasmic signaling molecules	
		Innexins		(87)
	4TM	Bestrophins	Cl^--selective (some are Ca^{2+}-activated)	(88)
	2TM (pentamers)	MscL	Nonselective (mechanosensitive, amphiphile-activated)	(89) (90)
	3TM (heptamers)	MscS	Nonselective (mechanosensitive)	(90)
Organellar membrane	6TM (tetramers)	RYR	Ca^{2+} (cation) selective (Ca^{2+}-activated)	(91)
		IP_3R	Ca^{2+} (cation) selective (IP_3- and Ca^{2+}-activated)	(92)
Outer membrane	β-barrels	Porins VDAC	Nonselective (except for some nutrients)	(93) (94)
Viral channels	1TM (tetramers)	M2	H^+ selective	(95)

transitions depicted in **Fig. 6** extend over distances that are much longer than the ions' mean free path, which means that one cannot use Eyring's transition state theory with a prefactor of k_BT/h to relate the measured/deduced rate constants to an underlying energy profile (111).]

Membrane potentials and single-channel currents

The rate of ion movement through a channel, the single-channel current (i), varies as a function of V_m and the channel's reversal potential, V_{rev}, defined as the membrane potential where $i=0$:

$$i = g \cdot (V_m - V_{rev}) \quad (3a)$$

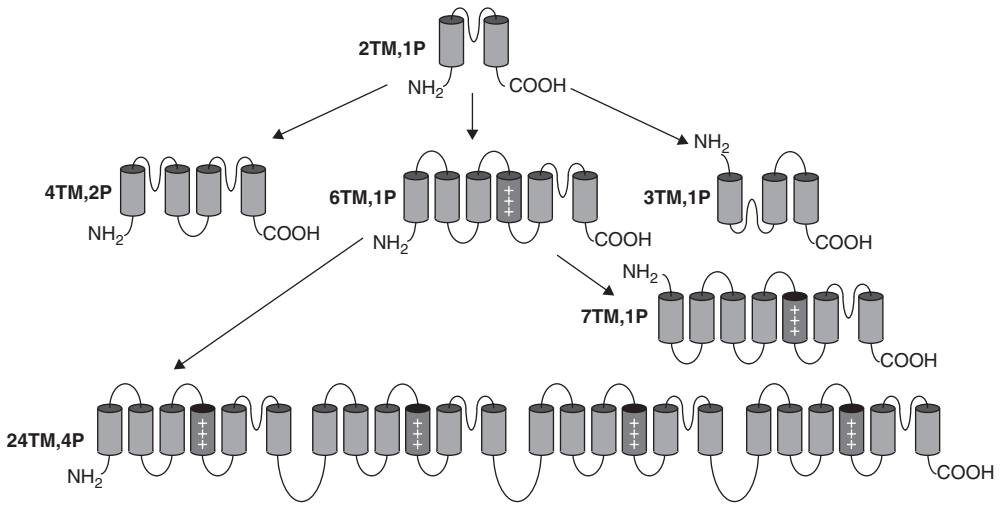

Figure 4 Schematic diagram that shows the TM segment organization for different pore-loop plasma membrane ion channels (listed in **Table 1a**). The ancestral 2TM,1P motif (exemplified by KcsA; **Fig. 3**) has evolved by gene duplication and the accretion of additional TM domains—and an inversion of transmembrane topology in the case of the 3TM,1P glutamate receptors.

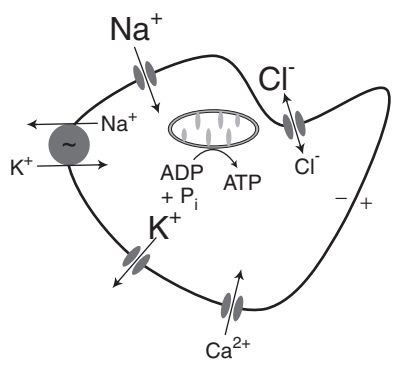

Figure 5 Schematic representation of a cell, with channels that are selective for Cl^-, Ca^{2+}, K^+, and Na^+ plus the Na^+,K^+-ATPase that maintains the ion distribution between the extracellular and intracellular compartments via an ATP-driven extrusion of Na^+ and uptake of K^+. The ATP is generated in the mitochondria by oxidative phosphorylation from ADP and inorganic phosphate (P_i). In resting cells, when no net charge movement is across the plasma membrane, the intracellular compartment is electrically negative relative to the extracellular compartment.

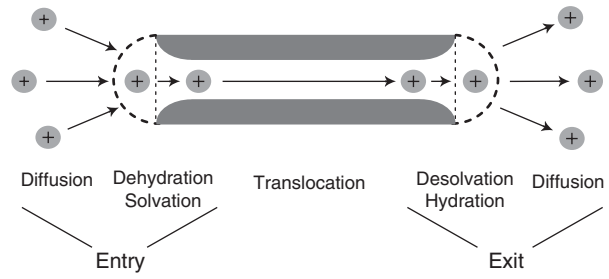

Figure 6 Schematic representation of ion movement through a bilayer-spanning channel. Ions diffuse to the channel entrance, where they undergo a dehydration and solvation by polar groups that line the pore wall, then they translocate through the pore in a diffusive barrier crossing for then to desolvate and rehydrate and finally diffuse into the bulk solution.

where g is the single-channel conductance, which varies as a function of channel type, the permeant ion concentration(s), and V_m—with most channels having conductances that range between 5 and 50 pS (see Ref. 1, Fig. 12.8). The total membrane current (I) that is carried across by all channels of a given type will be:

$$I = N \cdot i = N \cdot g \cdot (V_m - V_{\text{rev}}) = G \cdot (V_m - V_{\text{rev}}) \quad (3b)$$

where N denotes the number of *conducting* channels in the membrane and G denotes the total membrane conductance contributed by the channels in question. In the case of highly selective channels that catalyze the transmembrane movement of only a single ion type, V_{rev} becomes equal to the ion's equilibrium (or Nernst) potential E:

$$E = \frac{-k_B T}{z \cdot e} \cdot \ln\left\{\frac{C_i}{C_e}\right\} \quad (4)$$

where C_i and C_e denote the intracellular and extracellular ion concentrations, respectively. **Table 2** lists values of E for the four major ions involved in cellular electrical activity. (Strictly, E should be expressed in terms of the ion activities, but activity coefficients are neglected in Eq. 4 because the ionic strengths of the extracellular and intracellular solutions are similar.)

If only a single type of highly selective ion channels is found in the membrane, then the resting membrane potential—the time-invariant potential of a cell "at rest" when the net current across the membrane is 0—will be equal to the equilibrium potential for the ion in question. If the membrane is endowed with several different types of highly selective ion channels, then V_m becomes a weighted average of the equilibrium potentials for the different ions, for example:

$$V_m = \frac{G_{\text{Na}} \cdot E_{\text{Na}} + G_{\text{K}} \cdot E_{\text{K}} + G_{\text{Ca}} \cdot E_{\text{Ca}} + G_{\text{Cl}} \cdot E_{\text{Cl}}}{G_{\text{Na}} + G_{\text{K}} + G_{\text{Ca}} + G_{\text{Cl}}} \quad (5)$$

Table 2 Extracellular and intracellular ion concentrations and equilibrium potentials

Ion	Extracellular concentration (mM)	Intracellular concentration (mM)	Equilibrium potential (mV)
Na^+	145	~12	+67
K^+	4.5	~150	−94
Ca^{2+}	~1.5	~0.0001	+129
H^+	0.00004	~0.0001	−24
Cl^-	115	~10	−65
HCO_3^-	25	~10	−24

Ion concentrations in millimoles per liter of water. Values for mammals are modified from Ref. 104, tables 26–5 and 26–6. The $[Ca^{2+}]$ are the free ion concentrations; intracellular $[Cl^-]$ varies considerably among cell types, ranging from ~4 mM in skeletal muscle to ~80 mM in red blood cells. The equilibrium potentials were calculated for $T = 37^\circ C$ using the listed concentrations.

Table 3 Ion selectivities and reversal potentials for selected ion channels[a]

Channel type	Permeability ratio	V_{rev}
Voltage-dependent sodium channel (Na_v)	$P_K/P_{Na} < 0.1$	> +45
Epithelial sodium channel (ENaC)	$P_K/P_{Na} \approx 0.001$	+66
Voltage-dependent potassium channels (K_v)	$P_{Na}/P_K < 0.01$	< −86
Voltage-dependent calcium channels (Ca_v)	$P_{Na}/P_{Ca} \approx 0.0008$	+63
Cyclic nucleotide-activated channels (CNG)	$P_K/P_{Na} \approx 1.1$	+17
Nicotinic acetylcholine receptor (nAChR)	$P_K/P_{Na} \approx 1.1$	−4
$GABA_AR$	$P_{HCO3}/P_{Cl} \approx 0.4$	−58

[a]Modified from Ref. 1 (tables 14.2–6), except for $GABA_AR$, where P_{HCO_3}/P_{Cl} is, which is from Ref. 114.
V_{rev} was calculated using the ion concentrations in **Table 2** and the permeability ratios in **Table 3** and Eq. 6 ($T = 37^\circ C$) or, for channels permeable to both mono- and divalent cations, a generalized version of Eq. 6 (115).

where the subscripts denote different ions. In the resting cell, G_K usually is much larger than the membrane conductances for other ions, so V_m will be close to E_K. In general, V_m and the cell functions that are regulated by V_m will vary as a function of the relative contributions of the different ions to the membrane conductance, $G_m = G_{Na} + G_K + G_{Ca} + G_{Cl} + \ldots$

Ion selectivity

Some ion channels, notably potassium and calcium channels, have remarkable ion selectivity, to the extent that one in many cases can neglect the movement of other ions. Most ion channels, however, are measurably permeable to several different ions, in which case their V_{rev} will vary as function of the permeant ion concentrations and the ions' relative permeabilities, as expressed by the so-called Goldman–Hodgkin–Katz equation (1), which for monovalent cations becomes:

$$V_{rev} = \frac{-k_B T}{e} \cdot \ln\left\{\frac{P_{Na}[Na^+]_i + P_K[K^+]_i}{P_{Na}[Na^+]_e + P_K[K^+]_e}\right\} \quad (6)$$

where P_{Na} and P_K denote the permeability coefficients for Na^+ and K^+, respectively, and the ratio P_{Na}/P_K is a measure of the channel's ability to discriminate between Na^+ and K^+. [Eq. 6 is widely used to quantify ion channels' selectivity, but it is an approximation that is valid only in special cases (112, 113); the permeability ratios, for example, P_{Na}/P_K, therefore should be regarded as empirical descriptors of a channel's ion selectivity.]

Table 3 lists representative values for the permeability coefficients of different channels types (both selective and nonselective cation permeable channels as well as anion permeable channels) and V_{rev} calculated using the ion concentrations in **Table 2** and Eq. 6. Hille (Ref. 1, chapter 14) should be consulted for a more extensive compilation and discussion.

Comparing the ion equilibrium potentials in **Table 2** with the channel reversal potentials in **Table 3** shows that a channel's reversal potential may differ substantially from the name-giving ion's equilibrium potential.

Membrane potential changes and transmembrane charge movements

Ion channels provide the structural underpinnings for the ion movements that underlie the rapid changes in membrane potential that are involved in the electrical signaling (the action potentials) in nerve and muscle cells (4) and in many other cell functions. **Fig. 7** shows the time course of the potential change during an action potential together with the time courses of the underlying changes in the membrane conductances to Na^+ and K^+. The initial depolarization is due to the rapid activation (opening) of Na_V channels; the later repolarization is because of the slower activation of K_V channels and deactivation and inactivation of Na_V channels, which eventually lead to a transient hyperpolarization (because G_K becomes so large that V_m approaches E_K).

Because cell membranes have a finite capacitance C_m [$C_m = A \cdot C_{sp}$, where C_{sp} is the specific membrane capacitance, ~0.9

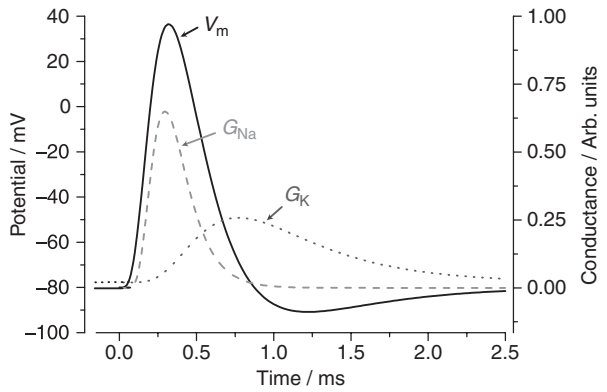

Figure 7 Schematic time course of an action potential. The initial rising, or depolarizing, phase of the action potential is because of an increase in the conductance to Na^+ (G_{Na}). At the peak, the membrane potential approaches the Na^+ equilibrium potential. The later falling, or repolarizing, phase is due to an increase in the conductance to K^+ (G_K) and a decrease in G_{Na}. Toward the end of the action potential, the membrane potential approaches E^K because (G_K) is much higher than at rest.

$\mu F/cm^2$ (116, 117) and A the membrane area], these changes in membrane potential require only a small transmembrane charge movement ΔQ:

$$\Delta Q = C_m \cdot V_m \tag{7}$$

The net ion movements that underlie electrical signaling are minute: according to Eq. 7, a 100 mV change in V_m is associated with a net transmembrane ion movement of $\sim 6 \cdot 10^3$ ions/μm^2. For a spherical cell of radius 10 μm, the resulting change in the (volume-averaged) intracellular ion concentration would be $\sim$3 μM; for a nerve fiber of radius 0.5 μm, the intracellular concentration change would be $\sim$40 μM. The actual ion movements are about three-fold higher than the minimum value estimated using Eq. 7 (Ref. 118, pp. 45–46); even then, the changes in V_m that underlie the action potential usually will not cause chemically measurable changes in the intracellular or extracellular ion concentrations (as can be seen by comparing these concentration changes with the concentrations listed in **Table 2**). The major exceptions to this rule are: ions that are present at very low concentrations, such as cytoplasmic Ca^{2+} and H^+ [where the concentration changes, however, will be attenuated by cytoplasmic Ca^{2+} and H^+ buffers (119, 120)]; cell processes with a very high surface-area-to-volume ratio (e.g., see Ref. 121); and tissues with small extracellular spaces, where K^+ and H^+ can accumulate (98, 122, 123).

Electrical signaling results from controlled changes in the cell membrane's permeability (conductance) to different ions, which result in changes in V_m (cf. Eq. 5). The steady-state V_m per se is determined only by the relative conductances for the different ions (Eq. 5), but the time course of changes in V_m (such as during an action potential; **Fig. 7**) depends on the absolute membrane conductances (channel densities in the membrane). Approximating the change in V_m as an exponential transient with a time constant ($\tau = C_m/G_m$) of 10 μs, the membrane conductance will need to be $\sim 10^{-9}$ S/μm^2—corresponding to a channel density of $\sim$100/μm^2 (for channels with g = 10 pS)—meaning that a 100 mV change in V_m is associated with a net movement of only $\sim$200 ions/channel. Although ion channels have very high turnover numbers, the actual number of ions that move through a channel during an action potential is quite small.

Bulk ion movement

In addition to the importance of ion channels for cellular electrical activity, where one to a first approximation can disregard the bulk movement of ions, ion channels serve two additional functions. First, as noted above, in the case of Ca^{2+}, the channel-mediated Ca^{2+} movement may cause a measurable increase in the cytoplasmic [Ca^{2+}], which in turn regulates many different cell functions, including neurotransmitter release, enzyme and hormone secretion, muscle contraction, and gene activation (124). Second, the bulk absorption of solutes across epithelial membranes depends not only on the presence of ATP-dependent ion pumps, in particular the Na^+,K^+-ATPase, but also on the ion channels imbedded in the apical and basolateral membranes (125).

Channel Gating

Ion channel function is tightly regulated to maintain normal function, such as the generation of action potentials, and to avoid compromising the cellular electrolyte, volume, and overall metabolic balance. Channel function is regulated by different stimuli, for example: membrane potential changes, usually positive-going (also called depolarizing) potential changes; changes in the concentration of extracellular (neuro)transmitters or intracellular messengers, which may be inorganic ions such as H^+, Ca^{2+}, and Zn^{2+}; light adsorption; covalent modification, such as phosphorylation or dephosphorylation; a mechanical perturbation; and so forth.

Gating transitions

Channel gating usually involves transitions among several different channel states (**Fig. 8**): closed, open, and inactivated (in the case of voltage-dependent channels) or desensitized (in the case of ligand-activated channels).

Channel inactivation and desensitization describe transitions from conducting to nonconducting channel states at a sustained membrane depolarization or ligand concentration (1). The inactivated or desensitized states are unresponsive to more depolarization or increases in the activating ligand concentration. The physiological effect of inactivation and desensitization is to reduce ion movement during excessive stimulation.

Whatever the gating mechanism or channel type, the total number of active, i.e., conducting or open, channels (N) in a membrane will be equal to the total number of channels of that type in the membrane (N_{tot}) times the probability the channel is in the open state (W_O):

$$N = N_{tot} \cdot W_O \tag{8}$$

where W_O will vary as a function of the stimulus strength and time (after a given stimulus is applied). (Because the letter P

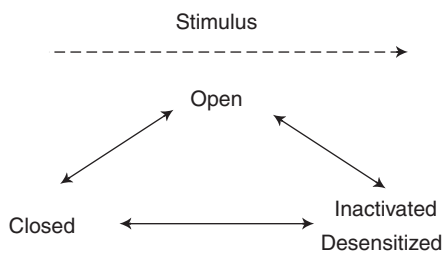

Figure 8 The major transitions in channel gating. An activating stimulus leads to channel opening; the stimulus also causes the channel to enter one or more nonconducting states, which differ from the resting closed states and are denoted inactivated states, (in the case of voltage-dependent channels) or desensitized states (in the case of ligand-activated channels).

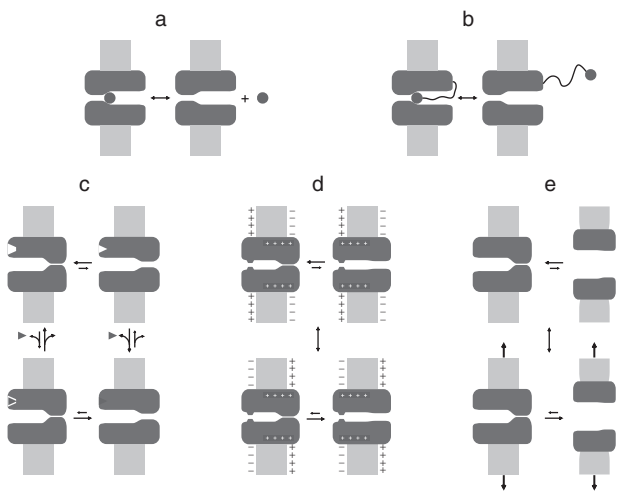

Figure 9 Ion channel gating mechanisms. In each diagram, the extracellular solution is to the left and the intracellular solution is to the right of the channel and its host bilayer. The horizontal arrows in panels A and B denote the reversible transition between the two states; the vertical arrows in the middle of panels D and E denote transitions between the upper and lower equilibria. a) Block by a small molecule that can enter the pore, where it binds to occlude the lumen and thereby block ion movement. b) Block by a covalently attached channel domain that is able to enter the pore to occlude the lumen and block ion movement. c) Ligand activation by a (neuro)transmitter, where the channel exists in two states, nonconducting and conducting, and the equilibrium distribution between the two states is shifted when the activating ligand binds. d) Voltage activation, changes in the membrane potential (cf. the upper and lower panels) shifts the distribution between different positions of a voltage sensor (indicated by the bar with positive charges); the channel is in the conducting state when the voltage sensor is in the outward position. e) Mechanical activation, where an increase in membrane tension (indicated by heavy arrows in the middle of each membrane) shifts the distribution between a closed state and an open state that has a greater cross-sectional area.

is used to denote permeability, we use the letter W, for the German "Warscheinlichkeit," to denote probabilities.)

Channel gating involves transitions among several closed (resting nonconducting) states, one or more open (conducting) states and, maybe, one or more nonconducting inactivated or desensitized states (**Fig. 8**). The principles underlying channel gating can be understood by considering just transitions between two states, closed (C) and open (O), where the stimulus shifts the distribution between the C and O states (**Fig. 9**).

Channel block

In the simplest case (**Fig. 9a**), channel gating involves a (voltage-dependent) block of the ion permeable pore (126). The blocking molecule (B) binds at a site in the pore lumen, and, because electrical potential varies along the pore, B's dissociation constant (K_B) will vary as a function of V_m. In the case of an impermeable cytoplasmic blocker that cannot pass through the pore, the blocker's reaction with the channel can be described as:

$$\mathrm{O} + \mathrm{B} \rightleftarrows \mathrm{OB};\ k_B = \frac{[\mathrm{O}] \cdot [\mathrm{B}]}{[\mathrm{OB}]};$$
$$k_B(V_m) = k_B(0) \cdot \exp\left\{-\frac{z \cdot e \cdot (1-\delta) \cdot V_m}{k_\mathrm{B} T}\right\} \qquad (9)$$

where O and OB denote the conducting (open) and nonconducting (obstructed) state, z the blocker's valence, and δ the fraction of V_m that falls between the extracellular solution and the site where the blocker binds. The probability that the channel is conducting thus becomes:

$$W_\mathrm{O} = \frac{k_B(0) \cdot \exp\left\{-\frac{z \cdot e \cdot (1-\delta) \cdot V_m}{k_\mathrm{B} T}\right\}}{K_B(0) \cdot \exp\left\{-\frac{z \cdot e \cdot (1-\delta) \cdot V_m}{k_\mathrm{B} T}\right\} + [\mathrm{B}]} \qquad (10)$$

This mechanism describes many aspects of the rectification in inward rectifying potassium channels (Kir; cf. **Table 1a** and **Fig. 3**), where cytoplasmic amines such as spermine and spermidine can enter the pore to block ion movement (49). It also is involved in the local anesthetic-induced block of $\mathrm{Na_V}$ by (positively) charged anesthetics, although local anesthetics also alter other channel properties (127).

In a more elaborate version of this gating mechanism (**Fig. 9b**), the blocking particle is covalently attached to the channel but still can enter the pore to block ion movement. This mechanism is observed in the fast inactivation of voltage-dependent channels (128).

Ligand activation

In the case of ligand-activated channels (**Fig. 9c**), such as the Cys-loop receptors (cf. **Table 1a** and ~~the section titled~~ "Cys-Loop Channels"), an agonist (A) binds with different affinities to the C and O states, and the difference in ligand binding energy to the two states shifts the O/C distribution (129–131):

$$\frac{L^{\mathrm{CA}\rightarrow\mathrm{OA}}}{L^{\mathrm{C}\rightarrow\mathrm{O}}} = \frac{K_\mathrm{A}^\mathrm{C}}{K_\mathrm{A}^\mathrm{O}} = \exp\left\{-\frac{\Delta G_\mathrm{A}^\mathrm{O} - \Delta G_\mathrm{A}^\mathrm{C}}{k_\mathrm{B} T}\right\} \qquad (11)$$

where $L^{\mathrm{C}\rightarrow\mathrm{O}}$ and $L^{\mathrm{CA}\rightarrow\mathrm{OA}}$ denote the equilibrium constant for the interconversion between C and O and between their agonist-bound states CA and OA, respectively; K_A^C and K_A^O denote the dissociation constants for agonist binding to C and O; and $\Delta G_\mathrm{A}^\mathrm{C}$ and $\Delta G_\mathrm{A}^\mathrm{O}$ denote the standard free energies for

agonist binding to C and O, respectively. The dose-response curve for channel activation by the agonist thus becomes:

$$W_{\mathrm{O}}([\mathrm{A}]) = \frac{W_{\mathrm{O}}(0) \cdot EC_{50} + W_{\mathrm{O}}(\infty) \cdot [\mathrm{A}]}{EC_{50} + [\mathrm{A}]} \qquad (12)$$

where $W_{\mathrm{O}}(0)$ and $W_{\mathrm{O}}(\infty)$ denote the channel open probabilities in the limits [A] $\rightarrow$ 0 and [A] $\rightarrow \infty$, respectively:

$$W_{\mathrm{O}}(0) = \frac{L^{\mathrm{C}\rightarrow\mathrm{O}}}{L^{\mathrm{C}\rightarrow\mathrm{O}}+1};\; W_{\mathrm{O}}(\infty) = \frac{L^{\mathrm{CA}\rightarrow\mathrm{OA}}}{L^{\mathrm{CA}\rightarrow\mathrm{OA}}+1} \qquad (13)$$

and

$$\begin{aligned} EC_{50} &= \frac{L^{\mathrm{C}\rightarrow\mathrm{O}}+1}{L^{\mathrm{CA}\rightarrow\mathrm{OA}}+1} \cdot K_{\mathrm{A}}^{\mathrm{C}} \\ &= \frac{L^{\mathrm{C}\rightarrow\mathrm{O}}+1}{L^{\mathrm{C}\rightarrow\mathrm{O}} \cdot K_{\mathrm{A}}^{\mathrm{C}} + K_{\mathrm{A}}^{\mathrm{O}}} \cdot K_{\mathrm{A}}^{\mathrm{C}} \cdot K_{\mathrm{A}}^{\mathrm{O}} \end{aligned} \qquad (14)$$

is the agonist concentration at which

$$W_{\mathrm{O}}(EC_{50}) = (W_{\mathrm{O}}0) + W_{\mathrm{O}}(\infty))/2,$$

which differs from both $K_{\mathrm{A}}^{\mathrm{C}}$ and $K_{\mathrm{A}}^{\mathrm{O}}$ [cf. (132)].

The gating of ligand-activated channels usually is more complicated than summarized here, e.g. (68, 133). First, channel activation usually involves binding of two (or more) agonist molecules; second, sustained increases in the agonist concentration promote an additional gating transition—to a nonconducting, desensitized state (cf. **Fig. 8**)—which is associated with additional conformational transitions [(68, 133, 134); see also Ligand-Operated Ion Channels].

Voltage-dependent gating

In the case of voltage-activated channels (**Fig. 9d**), such as the 6TM,1P K_V channels or the 24TM,4P Na_V or Ca_V channels (cf. **Table 1a**, ~~the section titled~~ "*Pore-Loop Channels*, and **Fig. 4**), changes in V_m are sensed by the translocation of charges or reorientation of dipoles by the electric field (135, 136). In the case of the voltage-dependent K_V, Na_V, and Ca_V channels, the charge translocation develops from a reorientation of the positively charged S4 segment (**Fig. 4**), which leads to an effective transmembrane transfer of about three charges per subunit or domain (137, 138). This charge movement is coupled to conformational changes that cause the channel to open when the membrane is depolarized (cf. 139, 140). Atomic resolution structures are available for several K_V channels (141–143), but the mechanism underlying the charge movement has not been fully clarified (136).

Although the channels are activated (opened) by depolarizing the membrane, the C $\leftrightarrow$ O equilibrium usually is strongly shifted toward O at $V_m = 0$ mV (139); that is, the depolarization removes a "stimulus" that normally keeps the channels closed.

The voltage-dependent shift in the C $\leftrightarrow$ O equilibrium is given by:

$$\frac{L^{\mathrm{C}\rightarrow\mathrm{O}}(V_m)}{L^{\mathrm{C}\rightarrow\mathrm{O}}(0)} = \exp\left\{\frac{z_a \cdot e \cdot V_m}{k_{\mathrm{B}}T}\right\} \qquad (15)$$

where $L^{\mathrm{C}\rightarrow\mathrm{O}}(V_m)$ denotes the voltage-dependent C $\leftrightarrow$ O equilibrium constant, and z_a denotes the gating valence (the charge movement associated with the C $\rightarrow$ O transition; z_a is positive for an outward movement of positive charge). The probability of the channel being open at a given voltage, V_m, thus becomes:

$$\begin{aligned} W_{\mathrm{O}}(V_m) &= \frac{L^{\mathrm{C}\rightarrow\mathrm{O}}(0)}{L^{\mathrm{C}\rightarrow\mathrm{O}}(0) + \exp\{-z_a \cdot e \cdot V_m/k_{\mathrm{B}}T\}} \\ &= \frac{1}{1+\exp\left\{-z_a \cdot e \cdot (V_m - V_{1/2})/k_{\mathrm{B}}T\right\}} \end{aligned} \qquad (16)$$

where $V_{1/2}$ is the potential at which $W_0(V_{1/2}) = 0.5$ (the midpoint potential for channel activation),

$$V_{1/2} = \frac{k_{\mathrm{B}}T}{z_a \cdot e}\ln\left\{L^{\mathrm{C}\rightarrow\mathrm{O}}(0)\right\} \qquad (17)$$

As is the case for the ligand-activated channels, the gating of voltage-activated channels is more complicated than summarized here: first, there usually are multiple closed states in the activation (139, 140, 144); second, sustained depolarizations tend to promote transitions to one or more non-conducting, inactivated states (**Fig. 8**) (cf. 128).

Voltage- and ligand-activated channels

The distinction between voltage- and ligand-activation is not always clear-cut. The function of many (if not all) voltage-dependent channels can be modulated by small molecules (including drugs and toxins) (145), see also ~~the section titled~~ "Regulation of Channel Gating", and the gating of ligand-activated channels can be modulated by changes in V_m (146). The most striking example of this dual regulation is the BK Ca^{2+}-activated potassium channels, which are both voltage- and ligand-activated (63, 147, 148).

Mechanosensitive channels

Mechanosensitive channels are activated by various mechanical stimuli (89, 149–151), including changes in membrane tension (σ), which will shift the C $\leftrightarrow$ O equilibrium when the cross-sectional channel area differ for the C and O states:

$$\frac{L^{\mathrm{C}\rightarrow\mathrm{O}}(\sigma)}{L^{\mathrm{C}\rightarrow\mathrm{O}}(0)} = \exp\left\{\frac{\Delta A \cdot \sigma}{k_{\mathrm{B}}T}\right\} \qquad (18)$$

where $L^{\mathrm{C}\rightarrow\mathrm{O}}(\sigma)$ denotes the tension-dependent, C $\leftrightarrow$ O equilibrium constant, and $\Delta A = A_{\mathrm{O}} - A_{\mathrm{C}}$ where A_{O} and A_{C} denote the cross-sectional area of the open and closed state, respectively. The probability of the channel being open at a given tension, σ, thus becomes:

$$\begin{aligned} W_{\mathrm{O}}(\sigma) &= \frac{L^{\mathrm{C}\rightarrow\mathrm{O}}(0)}{L^{\mathrm{C}\rightarrow\mathrm{O}}(0) + \exp\{-\Delta A \cdot \sigma/k_B T\}} \\ &= \frac{1}{1+\exp\left\{-\Delta A \cdot (\sigma - \sigma_{1/2})/k_B T\right\}} \end{aligned} \qquad (19)$$

where $\sigma_{1/2}$ is the membrane tension at which $W_O(\sigma_{1/2}) = 0.5$:

$$\sigma_{1/2} = -\frac{k_B T}{\Delta A} \cdot \ln\left\{L^{C\rightarrow O}(0)\right\} \qquad (20)$$

As is the case for ligand- and voltage-activated gating, the gating of mechanosensitive channels is more complicated than summarized here: changes in bilayer tension will cause changes in bilayer thickness (152), which in their own right may alter channel gating as has been demonstrated in the case of the gramicidin channel monomer ↔ dimer equilibrium (153); there usually are multiple closed, or intermediate conductance, states in the activation path (154); and sustained stretch can promote transitions to one or more nonconducting, desensitized states [**Fig. 8**; cf. (52); see also Mechanosensitive Channels].

Other mechanisms

Channel function also may be regulated by channel insertion into, or retrieval from, the target membrane—mediated by fusion, or budding, of vesicles in which the channels are incorporated in the vesicle membrane (77). These regulatory mechanisms will not be discussed more here (see also ''Dynamics of Cell Membranes'' and ''Membrane Trafficking'').

Regulation of Channel Gating

Ion channels, as other enzymes, are functionally regulated by covalent modification of the constituent protein [e.g., by phosphorylation (1) or oxidation (155)]; by small molecules that bind reversibly to the channel protein [(156, 157); see also Ligand-Operated Ion Channels and Drug Design Strategies for Targeting G-Protein-Coupled Receptors]; and by amphiphiles that bind the surrounding host bilayer (158, 159). The mechanisms that underlie the regulation of globular proteins, therefore, also pertain to the regulation of ion channels (membrane proteins) (cf. Ref. 160, chapters 7–10).

Ion channels are special

Ion channels, however, are subject to additional regulatory mechanisms that do not pertain to globular proteins and which will be the focus here. These novel regulatory mechanisms arise from a key difference between ion channels (and other membrane proteins) and globular proteins, namely that the channels are inserted into and span the membranes' lipid bilayer. This is important because the conformational changes that underlie ion channel function involve the channels' TM domain and, therefore perturb the packing of the adjacent lipids [(134, 161, 162); cf. **Fig. 10**], which incurs an energetic cost (163).

Hydrophobic matching and hydrophobic coupling

The hydrophobic interactions between the channel's hydrophobic TM domain and the host lipid bilayer cause the bilayer to adapt to the channel's exterior, an adaptation called *hydrophobic matching* (164); channel conformational transitions that involve

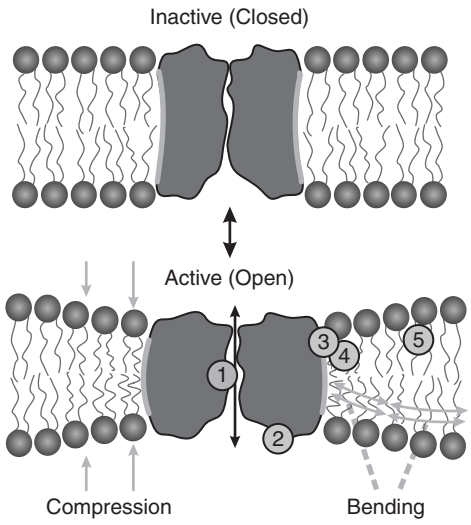

Figure 10 Schematic representation of different, nonexclusive mechanisms by which one can regulate ion channel function. For simplicity, the conformational change underlying the C → O is illustrated as a decrease in the length of the channel's hydrophobic TM domain, which is associated with a local deformation of the bilayer hydrophobic core (compression and bending of the two bilayer leaflets). 1) A drug may bind in, or very close to, the pore to block ion movement, 2) a drug may bind to a site wholly formed by the protein to either inhibit or potentiate the channel function by altering the free energy difference between different channel states, 3) an amphiphilic drug may bind specifically to a site composed of both the protein and the bilayer lipids, in which case its effects can involve also changes in the bilayer deformation energy associated with channel conformational changes, 4) an amphiphilic drug may accumulate nonspecifically at the protein/bilayer interface to alter local lipid packing and thereby alter the bilayer deformation energy contribution to channel's conformational changes, and 5) an amphiphilic drug may adsorb at the lipid bilayer/solution interface to alter the lipid bilayer material properties and thereby the bilayer deformation energy associated with channel conformational changes.

the channel/bilayer interface will alter this local bilayer perturbation, which incurs an energetic cost (165). For example, a transition between two different channel states, a closed (C) and an open (O) state, will be associated with a local bilayer deformation (**Fig. 10**). The total free energy change for the protein conformational change $\Delta G_{tot}^{C\rightarrow O}$ therefore may be described as:

$$\Delta G_{tot}^{C\rightarrow O} = \Delta G_{prot}^{C\rightarrow O} + \Delta\Delta G_{bilayer,local}^{C\rightarrow O} + \Delta\Delta G_{bilayer,bulk}^{C\rightarrow O} \qquad (21)$$

where $\Delta G_{prot}^{C\rightarrow O}$ is the energetic contribution from the protein conformational per se (not related to the bilayer perturbation), $\Delta\Delta G_{bilayer,local}^{C\rightarrow O}$ is the energetic cost of the changes in local lipid packing ($\Delta\Delta G_{bilayer,local}^{C\rightarrow O} = \Delta G_{bilayer,local}^{O} - \Delta G_{bilayer,local}^{C}$, where $\Delta G_{bilayer,local}^{C}$ and $\Delta G_{bilayer,local}^{O}$ denote the energetic cost of local lipid packing at the channel/bilayer interface for C and O, respectively), and $\Delta\Delta G_{bilayer,bulk}^{C\rightarrow O}(= \Delta G_{bilayer,bulk}^{O} - \Delta G_{bilayer,bulk}^{C})$ is the corresponding energetic cost of the bulk bilayer perturbation (166). The equilibrium distribution between

the two protein conformations is given by (165, 167):

$$\frac{[O]}{[C]} = \exp\left\{-\frac{\Delta G_{tot}^{C\to O}}{k_B T}\right\} = \exp\left\{-\frac{\Delta G_{prot}^{C\to O} + \Delta\Delta G_{bilayer,local}^{C\to O} + \Delta\Delta G_{bilayer,bulk}^{C\to O}}{k_B T}\right\} \quad (22)$$

The latter two energetic contributions vary as a function of the bilayer material properties [thickness, lipid intrinsic curvature, and the associated elastic moduli (166)]; that is, there is *hydrophobic coupling* is between the bilayer material properties and the function of bilayer-embedded proteins (ion channels) (167).

Mechanisms of Drug-Induced Ion Channel Modulation

Small molecules (e.g., drugs) can regulate ion channel function by at least five different mechanisms [(158); cf. **Fig. 10**]. (Specific examples of each of these mechanisms are listed in **Table 4**.) The molecules may bind in—or near—the channel pore to physically occlude the lumen and block ion movement (see mechanism #1 in **Fig. 10** and ~~the section titled~~ "Channel Block"). The molecules may regulate ion channel gating allosterically by binding to sites that are formed by the protein itself (as a conventional ligand binding site) to alter the $\Delta G_{prot}^{C\to O}$ contribution to $\Delta G_{tot}^{C\to O}$ (see mechanism #2 in **Fig. 10** and ~~the section titled~~ "Ligand Activation"). These two mechanisms are general and apply also to globular proteins.

Because many drugs are amphiphiles [being hydrophobic and yet having some aqueous solubility (180, 181)], they will adsorb to the host lipid bilayer in addition to binding to the target channel (membrane protein). This means that they can regulate channel function by additional allosteric mechanisms. First, they can bind at the channel/bilayer interface to alter the $\Delta\Delta G_{bilayer,local}^{C\to O}$ (and also $\Delta G_{prot}^{C\to O}$) contribution to $\Delta\Delta G_{tot}^{C\to O}$ (mechanism #3 in **Fig. 10**). Second, because the lipid bilayer material properties vary when amphiphiles adsorb to the bilayer/electrolyte interface (182), they can accumulate in the bilayer near the channel/bilayer interface and alter the $\Delta\Delta G_{bilayer,local}^{C\to O}$ contribution to $\Delta G_{tot}^{C\to O}$ (mechanism #4 in **Fig. 10**), and they can adsorb to the bulk bilayer and alter the $\Delta\Delta G_{bilayer,bulk}^{C\to O}$ contribution to $\Delta G_{tot}^{C\to O}$ (mechanism #5 in **Fig. 10**).

The common feature among mechanisms #2–#5 in **Fig. 10** is that ligand binding, to the protein or the bilayer/solution interface, alters the contributions to ΔG_{tot}^{0} for the conformational transitions between different channel states (cf. Eq. 22). At one extreme, mechanism #2 involves changes in $\Delta G_{prot}^{C\to O}$, a type of regulation that is shared by other (membrane-associated or water-soluble) enzymes and can be described by standard theories of ligand-receptor interactions (cf. ~~the section titled~~ "Ligand Activation"). At the other extreme, mechanism #5 depends solely on changes in $\Delta\Delta G_{bilayer,bulk}^{C\to O}$, which are determined by the bilayer continuum material properties. Mechanisms #3 and #4 depend on changes in $\Delta\Delta G_{bilayer,local}^{C\to O}$ (and, in the case of mechanism #3, also $\Delta G_{prot}^{C\to O}$), which like $\Delta\Delta G_{bilayer,bulk}^{C\to O}$ are determined by the molecular composition of the bilayer immediately surrounding the channel. They also may involve local accumulation of molecules to diminish the energetic constraints on the local packing of lipid molecules adjacent to the channel—as deduced for polyunsaturated fatty acids (183). The key difference between these two mechanisms and mechanism #5 is that the magnitude of $\Delta\Delta G_{bilayer,local}^{C\to O}$ also depends on the protein "shape." The difference between mechanisms #3 and #4 is that the site of modulation in #3 is formed in part by the channel's TM domain, which means that specific binding of the modulator to this site may occur (as well as a contribution from $\Delta G_{prot}^{C\to O}$).

A given molecule may affect ion channel function by any combination of these mechanisms. Mechanism #2 is the conventional allosteric mechanism. (Some drugs, such as diazepam, exert their action by binding to a site on the target and potentiating (or inhibiting) the action of another drug, such as GABA on the $GABA_A$ receptor; we denote this mechanism #2, cf. Table 4. To the extent that $\Delta\Delta G_{bilayer,local}^{C\to O}$ or $\Delta\Delta G_{bilayer,bulk}^{C\to O}$ are significant, on the order of $k_B T$, mechanisms #3–#5 become important, and the lipid bilayer will be an allosteric regulator of ion channel (membrane protein) function (165). Only mechanisms #1 and #2, and to a lesser extent #3, depend on specific, stoichiometric interactions with the channel; only mechanism #5 is altogether nonspecific. A challenge in future drug development becomes to identify the relative contribution of each of these mechanisms to a potential drug's overall effects—for then to optimize for the desired action. In this context, it is important that drugs with high affinity for the lipid bilayer/solution interface, relative to sites #1–#3, may exert a significant part of their action through mechanisms #4 and #5, which make them promiscuous modulators of membrane protein function.

We finally note that molecules that reduce channel currents often are described as channel "blockers," whether or not the molecule in question actually enters the pore to occlude it. This is bad practice; the term blocker should be reserved only for mechanism #1, where there is a physical block or occlusion of the pore. Other molecules that reduce the channel-mediated ion movement are best described as channel "inhibitors." This terminology becomes important when a current-reduction that is potentiated by repeated or frequent channel activation (as often is the case) is described as a "use-dependent block"—with the implication that mechanism #1 is involved. Any combination of mechanisms "1" to "5" may cause such an observation [cf. (177)], which is better described as "frequency dependent inhibition."

Conclusion

Ion channels constitute an important group of membrane proteins and are involved in many different biological functions. Because ion channels generally are efficient catalysts, they must be tightly regulated by mechanisms that control the channels' surface density and by mechanism that regulate the functional state of each individual channel. In addition to their physiological importance, the high turnover numbers allow

Table 4 Specific examples of mechanisms #1-#5

Mechanism	Channel	Molecule	Reference
#1	Na_V	Tetrodotoxin	(168)
#1	Ca_V	ω-Conotoxins	(169)
#1	K_V	Tetraethylammonium	(170)
#2	Na_V	α- and β-Scorpion toxins	(145)
#2	nAChR	ACh	(171)
#2	$GABA_A$	GABA	(172)
#2'	$GABA_A$	Diazepam	(173)
#2'	nAChR	α-Bungarotoxin	(174)
#3	Kir	PIP_2	(175, 176)
#3	ENaC	PIP_2	(175, 176)
#3	TRPV	PIP_2	(175, 176)
#4 & #5	Na_V	Capsaicin	(177)
#4 & #5	$GABA_A$	Docosahexaenoic acid*	(178)
#4 & #5	CFTR	Butanedione monoxime	(179)

Mechanism #2′ denotes the case in which the binding of one compound alters the affinity for the native ligand (e.g., GABA for $GABA_A$ and Ach for AChR).
ABBREVIATIONS: PIP_2 = phosphatidylinositol(4, 5)bisphosphate.
*Docosahexanoic acid may exert its effect also through mechanism #3.

for high-resolution measurements at the single-molecule level, which allow for detailed studies of how membrane protein function can be modulated/regulated. Finally, ion channels can be regulated—in addition to the mechanisms that have been established for globular proteins—by mechanisms that are unique for membrane proteins.

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