From molecule to malady

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Ion channels are membrane proteins, found in virtually all cells, that are of crucial physiological importance. In the past decade, an explosion in the number of crystal structures of ion channels has led to a marked increase in our understanding of how ion channels open and close, and select between permeant ions. There has been a parallel advance in research on channelopathies (diseases resulting from impaired channel function), and mutations in over 60 ion-channel genes are now known to cause human disease. Characterization of their functional consequences has afforded unprecedented and unexpected insights into ion-channel mechanisms and physiological roles.

The cell membrane is a major barrier to ion movement, and specific proteins — the ion channels, transporters and pumps — have evolved to transport ions across it. Ion channels are gated pores that permit the passive flow of ions down their electrochemical gradients (Fig. 1). Ion pumps, by contrast, use the energy of ATP hydrolysis to transport ions against their electrochemical gradient. In between are the coupled transporters (antiporters and symporters), in which movement of one ion species against its electrochemical gradient is powered by the downhill movement of another.

This Insight focuses on ion channels. Over 340 human genes are thought to encode ion channels (see www.ensembl.org/Homo_sapiens/index.html). They have important roles in such diverse processes as nerve and muscle excitation, hormone secretion, cell proliferation, sensory transduction, learning and memory, regulation of blood pressure, salt and water balance, lymphocyte proliferation, fertilization and cell death[1]. Your ability to read and understand this page depends on the activity of ion channels in your eye and brain. Consequently, defects in ion-channel function often have profound physiological effects.

Because of their important functional roles, their membrane location, structural heterogeneity and the restricted tissue expression of some channel types, ion channels are attractive targets for drug therapy. Indeed, many existing drugs, such as local anaesthetics[2], sedatives[3], anti-anxiety agents[3], antidiabetic drugs (see the review in this issue by Nichols, p. 470) and even antiviral therapies[1,4], exert their effects by interacting with ion channels.

Our understanding of how ion channels function has been illuminated by recent breakthroughs in high-resolution structure determination and by studies of diseases that result from impaired channel function (the channelopathies). This review provides an introduction to ion-channel structure, a theme that is developed more fully in the subsequent reviews. It also provides an overview of channelopathies.

Structural considerations

Ion channels are composed of one or more pore-forming subunits, often in association with accessory subunits. Most channels conform to a common structural theme in which the central pore, through which the ions move, is formed by four or five transmembrane α-helices, which fit together like the staves of a barrel. In many channels, the pore-forming helices are contributed by separate subunits, so that the channel is tetrameric (Kir channels, for instance) or pentameric (Cys-loop receptors, Fig. 2). However, voltage-gated Ca^{2+} (Ca_V) and Na^+ (Na_V) channels are composed of a single subunit that contains four similar repeated domains, and some K^+ channels are dimers, each subunit being composed of two repeated domains (Fig. 2).

The largest class of ion channels possess a pore loop, a region of the protein that loops back into the membrane to form the selectivity filter that determines which ion species can permeate (Fig. 2). They are either tetramers, or monomers of four similar domains. The human ether-a-go-go (hERG) channels, inwardly rectifying K^+ (Kir) channels and glutamate receptor channels discussed in subsequent reviews belong to this class. All pore-loop channels are built on a common pattern, which suggests that they evolved from a primordial channel resembling

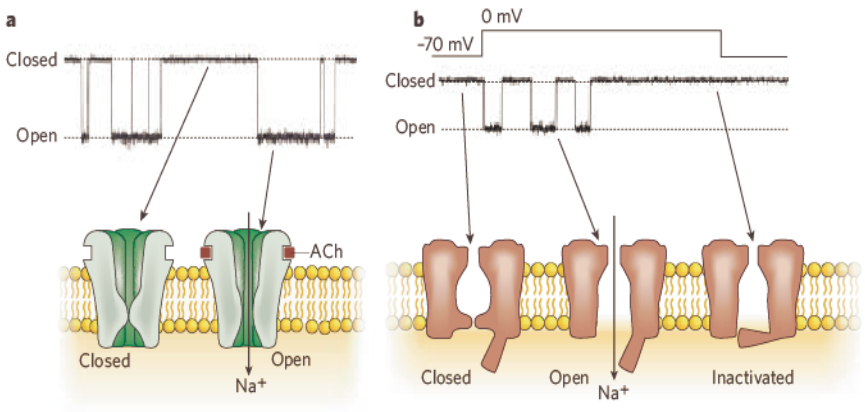

Figure 1 | Molecular nanoswitches. Schematics illustrating how ion channels open and close, with associated single-channel recordings. Opening and closing of the channel are random events, but the frequency with which they occur is influenced by, for example, ligand-binding (**a**) or transmembrane voltage (**b**). The transition rate between open and closed states is <10 μs. The flux rate through the pore when it is open is of the order of 10^7 ions per second; that mediated by the coupled exchangers is substantially smaller (see p. 484). Following opening, some voltage-gated channels enter an inactivated (non-conducting) state in which they are refractory to subsequent depolarization (**b**).

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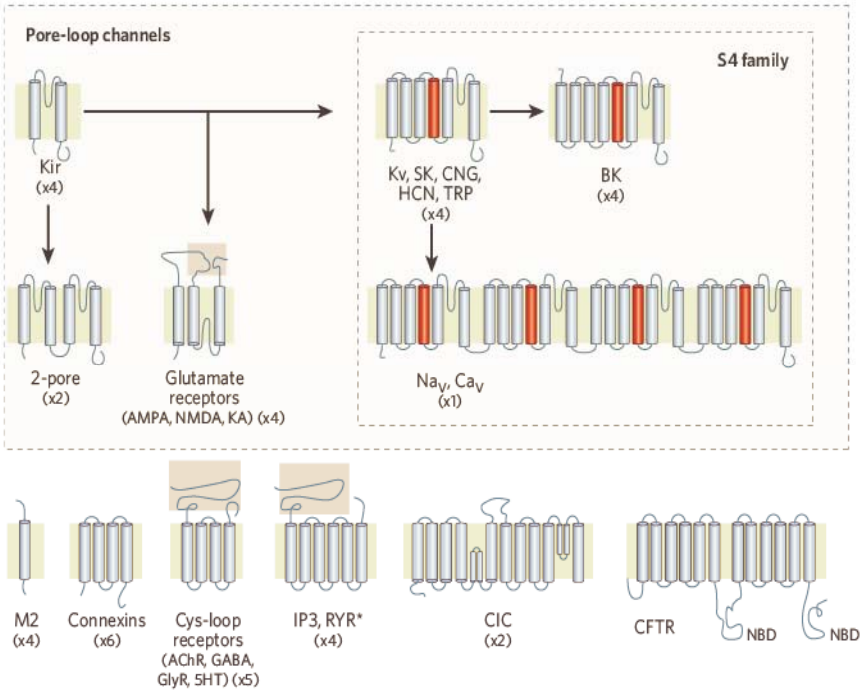


Figure 2 | Ion-channel classes. Predicted transmembrane topology of different types of channel. The numbers in parentheses refer to the number of subunits that make up the channel pore. The beige boxes indicate ligand-binding domains. The S4 domain is highlighted. Kir, inwardly rectifying K^+ channel. K_V, voltage-gated K^+ channel. Na_V, voltage-gated Na^+ channel. Ca_V, voltage-gated Ca^{2+} channel. CNG; cyclic-nucleotide-gated channel. HCN, hyperpolarization-activated channel. TRP, transient receptor potential channel. ClC, chloride channel. CFTR, cystic fibrosis transmembrane conductance regulator. IP3R, inositol trisphosphate receptor. RYR, ryanodine receptor. *The number of TMs of RYRs, whether they are evolutionarily related to IP3R, or if RYR and IP3R belong to the pore-loop family, or not, is unknown. The ClC channels have many helices that go only partway across the membrane and their transmembrane topology is not easily depicted (see p. 484).

the bacterial K^+ channels KcsA and KirBac[5,6]. The central core of these channels, which contains the ion-permeation pore, comprises two transmembrane helices (TMs) linked by a pore loop (Fig. 2). The Kir channels consist only of this core, and two-pore K^+ channels arose by tandem duplication. In the S4 subfamily, additional TMs (mostly four, but five in the case of the large-conductance Ca^{2+}-activated K^+ (BK) channels) are present at the amino-terminal end of the protein[7]. The fourth TM (S4) is adapted for voltage sensing and possesses several positively charged residues. Evolution has further developed the pore-loop theme by addition of extracellular and/or intracellular modules that act as docking sites for ligands, accessory subunits and other modulators. The glutamate receptors, for example, contain a pore-loop core, orientated in the membrane in the inverse direction, plus extracellular ligand-binding domains. Some K^+ channels have an added intracellular Ca^{2+}-binding site[8], and both cyclic-nucleotide-gated (CNG) channels[9] and hERG (see the review in this issue by Sanguinetti and Tristani-Firouzi, p. 463) have appropriated an intracellular cyclic-nucleotide-binding site. Like many proteins, therefore, ion channels are built on a modular theme. The tetrameric nature of most pore-loop channels contributes to ion-channel diversity, as closely related subunits can associate to form heteromeric channels with novel properties.

The non-pore-loop channels can be grouped into a variety of classes, which have no obvious evolutionary relationship (Fig. 2). The pentameric ligand-gated channels known as the Cys-loop receptors contain four TMs, the pore being formed by the second transmembrane helix in each of the five subunits. The neurotransmitter-binding site is found in the N terminus and, at least in some cases, may have been stolen from elsewhere. That of the acetylcholine receptor (AChR), for example, is also present in a separate water-soluble protein[10]. Interestingly, the Cys-loop receptors are found almost exclusively in metazoans (multicellular organisms), suggesting that they evolved only when cell–cell interactions such as those found in the nervous system became important[11].

Also discussed in this Insight are CFTR (see the review in this issue by Gadsby, Vergani and Csanády, p. 477) and the sulphonylurea receptor (SUR; see p. 470). These belong to the ATP-binding cassette (ABC) family of pumps that transport nutrients, drugs and solutes across the membrane using the energy of ATP hydrolysis. Uniquely, CFTR is a Cl^- channel, and its ATPase activity is used to drive the protein between open and closed conformations (see p. 477). The ABC protein SUR is neither a pump nor a channel but a channel regulator (see p. 470).

The ClC chloride channel family presents a conundrum, for it is now clear that some of these proteins are channels whereas others are transporters (see the review in this issue by Miller, p. 484). This example, along with that of CFTR and SUR (which 'should' be pumps), demonstrates that the classical distinction between channels and transporters is blurred and that the two types of ion-transport mechanism share common features. Understanding why certain family members function as transporters and others as channels will provide fundamental insights into the essential features that distinguish channels from transporters.

High-resolution structures of several ion channels, or their bacterial homologues, have been obtained, including those of Kir[6] and K_V[7] channels, the AChR Cys-loop receptor[13] (see the review in this issue by Sine and Engel, p. 448) and the Cl^- transporter ClC-ec1[12] (see also p. 484). Although the complete structure of CFTR has not yet been solved, the crystal structure of the first nucleotide-binding domain (involved in ATP hydrolysis) is known (see p. 477). High-resolution structures of the ligand-binding domains of glutamate receptors are also available (see the review in this issue by Mayer, p. 456). These structures, described in detail in the accompanying reviews, have illuminated our understanding of how ion channels work, as discussed below.

Properties of ion channels

The cardinal properties of ion channels are ion selectivity and gating. Selectivity refers to the ability of some channels to discriminate between ion species, allowing some to pass through the pore while excluding others. Gating is the process of transition between the open and closed states (Fig. 1).

Selectivity

Ion movement through a channel pore depends on the electrochemical gradient across the membrane and the permeability of the pore[14]. The largest pores, such as those of gap-junction channels[15,16], function like molecular sieves and discriminate only on the basis of size. In most cases, however, ion channels are very choosy about the ions they allow through. Some, such as AChR, permit flux of a variety of cations but exclude anions (see p. 448). They have a large water-filled pore (~6.5 Å in diameter) through which ions move in the (partially) hydrated state[13]. Rings of negatively charged residues in the pore exclude anions and facilitate cation flux. Conversely, anion-selective channels such as GABA and glycine receptors possess rings of positively charged residues.

Other channels, particularly those that belong to the pore-loop family, are even more discriminatory. Indeed, a re-entrant pore loop seems

to be a criterion for high selectivity as it is present in all highly selective channels (although the converse is not true — not all pore-loop channels are highly selective between ions of similar charge). K^+ channels are 100–1,000 times more permeable to K^+ than Na^+, despite the fact that the unhydrated Na^+ ion is smaller[14]. This exquisite discrimination takes place at the narrowest part of the pore, known as the selectivity filter. How this is achieved became crystal clear in 1998 when the structure of the bacterial K^+ channel KcsA was solved[5]. All K^+ channels possess an almost invariant GYG sequence that earlier functional studies had shown lies at the heart of the selectivity filter[17]. The X-ray structure revealed that the backbone carbonyls of the GYG residues line the pore and substitute for the waters of hydration, so that K^+ passes through the filter by slipping from one binding site to the next[5]. The carbonyl oxygens are too far apart to enable them to interact intimately with Na^+ ions, so that Na^+ is effectively excluded from the selectivity filter because the energy required to remove the waters of hydration is greater than that gained by interacting with the carbonyl oxygens.

Na_V and Ca_V channels probably use a different strategy to achieve selectivity. Although no structures are yet available, functional studies show that these pores are wider than that of K^+ channels (4–6 Å compared with 3 Å) and that the side chains face into the pore[14,18,19]. Both types of channel use negatively charged residues, and perhaps some backbone carbonyls as well, to coordinate the cation.

Gating

The opening and closing of most channels is regulated by biological signals (Fig. 1) such as binding of intracellular or extracellular ligands (ligand-gated channels), changes in membrane potential (voltage-gated channels), changes in temperature[20] or mechanical stress[21]. In many cases, gating is also influenced by biochemical reactions such as phosphorylation, a process known as modulation.

Structural studies have revealed that most channels are closed by a gate that acts as a physical barrier to ion movement. This is achieved in a variety of ways. The gate of the gap-junction channel acts like the iris diaphragm of a camera[15]. In pore-loop channels, the inner TMs that line the pore act as a hinged gate, bending outwards to open the channel[22,23]. Straightening of the helix and sliding of the intracellular ends together (at the helix bundle-crossing region) closes the pore. Inactivation of some K_V channels is produced by a tethered N-terminal blocker, which swings into the pore and physically plugs it[14,24]. All these mechanisms involve large-scale movements of the protein backbone, but in ClC channels the conformational change is apparently quite subtle and the rotation of a single amino acid is sufficient to block ion permeation (see p. 484).

A non-steric gating mechanism has been proposed for AChR channels[25], although the resolution of the channel structure is not yet good enough to confirm the idea. Rather than physical occlusion, the gate may be an energetic one that results from a 'hydrophobic wall' to ion permeation. In AChR, closure is accompanied by rotation of TM2, which brings hydrophobic leucine side chains into the pore[13]. It is argued that unhydrated ions are actually small enough to pass though the pore but they are prevented from doing so because the energetic cost of dehydration next to a 'greasy wall' is too high[25]. (Remember that ions normally move through AChR in the hydrated state.)

These two types of gate are physically distinct from the selectivity filter. However, there is accumulating evidence that in some channels the selectivity filter itself may act as a second gate, which governs the rapid closings (flickers) characteristic of many ion channels[26].

Voltage gating

Voltage-gated channels are opened or closed by changes in transmembrane voltage. A fundamental requirement for voltage sensing is the transfer of electrical charge through the membrane electric field in response to transmembrane voltage changes. This 'gating charge' can arise from charged residues on the protein, for example in the fourth transmembrane segment (S4) (see p. 463), or from charged activator-ligands binding to sites deep within the protein, as in the ClC channels (see p. 484). In the S4 family, where it has been most studied, voltage sensitivity arises from the movement of three to four positively charged residues in S4 within the voltage field (see p. 463). Although the detailed nature of the S4 movement is currently controversial, there is wide agreement on the identity of the gating charges.

Ligand gating

The basic principle of ligand gating is that the ligand must bind differently to the open and the closed conformations. If it binds more tightly to the open state, the ligand will be a channel activator, whereas if binding to the closed configuration is stronger, it will be an inhibitor. There are several other features common to all ligand-gated channels. First, the ligand-binding site is almost invariably located at the interface between two adjacent subunits or domains. As described in the accompanying reviews, this is true for the acetylcholine-binding site

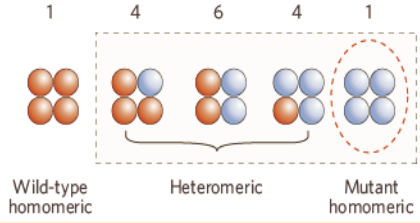
Box 1 | From malady to molecule and back again

New information on ion-channel structure affords unprecedented insight into how ion channels work and how disease-causing mutations achieve their functional effects. Conversely, identification of channel genes responsible for human disease has illuminated the relationship between channel structure and function. Kir6.2, the pore-forming subunit of the K_{ATP} channel (see p. 470), provides an example of this synergy.

The importance of the K_{ATP} channel in insulin secretion was established over 20 years ago[51], and fuelled the search for K_{ATP} channel mutations causing diabetes. Recent studies show that heterozygous mutations in Kir6.2 cause neonatal diabetes by impairing the ability of ATP to inhibit the channel[34,38] (see p. 470). This increases the K_{ATP} current, suppressing electrical activity and insulin secretion. Severe mutations that reduce ATP inhibition strongly also produce extra-pancreatic features such as epilepsy, developmental delay, muscle weakness and dysmorphic features (DEND syndrome)[34,38]. Presumably these mutations increase K_{ATP} currents enough to reduce electrical activity in nerve and muscle.

The severity of the disease is dictated by both the tetrameric nature of the channel and the functional consequence of the mutation[34]. Mutations that disrupt ATP binding usually cause only neonatal diabetes. This is because, although the channel has four ATP-binding sites (one per Kir6.2 subunit), binding of just one ATP shuts the pore[27]. Because mutant and wild-type subunits associate randomly, in the heterozygous state only one-sixteenth of channels will have strongly reduced ATP sensitivity (dotted line in the figure). By contrast, the more severe DEND mutations usually stabilize the intrinsic open state of the channel and reduce ATP block indirectly[38] (see p. 470). Because each subunit can contribute to gating of the tetrameric pore, heteromeric channels containing both wild-type and mutant subunits will be affected (that is, as many as 15 out of 16 channels in heterozygotes; dashed line in the figure). Consequently DEND mutations produce a greater reduction in ATP sensitivity and more severe disease[38]. Thus, Kir6.2 mutations illustrate how the tetrameric nature of the channel can result in very different phenotypes.

The different mechanisms of action of Kir6.2 mutations have important implications for therapy. In the past, neonatal diabetes was treated by insulin injection but many patients have now been successfully transferred to sulphonylurea tablets (which block K_{ATP} channels)[34] (see p. 470). However, these drugs (like ATP) are less effective on DEND mutations, which stabilize the channel open state. So the choice of therapy is dictated by the functional properties of the channel.



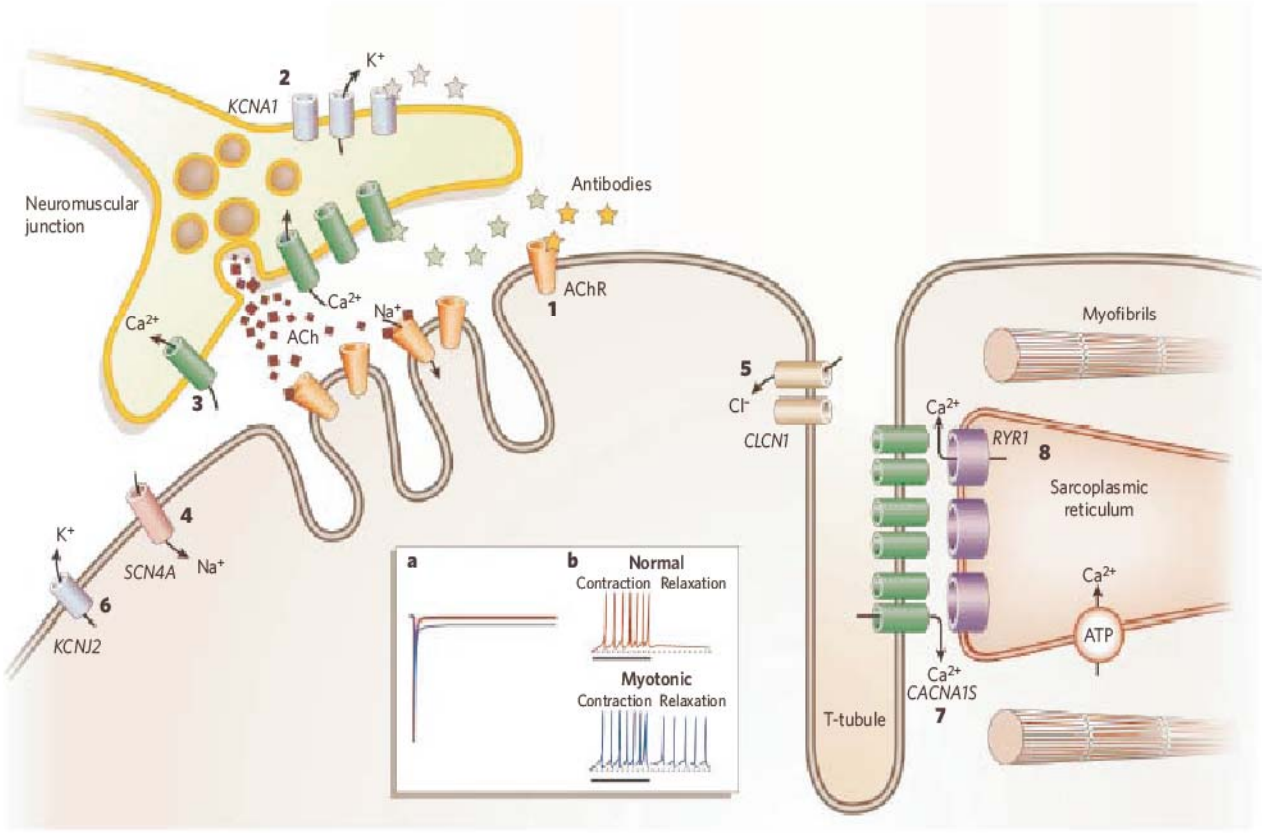


Figure 3 | Skeletal muscle channelopathies. **a**, Schematic of the motor nerve terminal, the neuromuscular junction and a skeletal muscle fibre, showing ion channels (numbered 1–8) whose mutation leads to disease (Tables 1 and 2). Mutations in several AChR subunits (1) cause myasthenia (muscle weakness). Autoimmune channelopathies are indicated by binding of antibodies (stars), which leads to internalization and downregulation of channel number. Loss of presynaptic K^+-channel function ($K_V1.1$, *KCNA1*) (2) leads to increased transmitter release and enhanced muscle contraction, whereas downregulation of presynaptic Ca^{2+} channels (3) or AChR (1) function causes myasthenia, by preventing neurotransmitter release, or binding, respectively. Gain-of-function mutations in the muscle Na^+ channel (4, *SCN4A*), and loss-of-function mutations in ClC channels (5), cause hyperexcitability and myotonia, while loss-of-function mutations in Kir1.1 (6) cause hyperexcitability and myotonia. Mutations in muscle Ca_V channels (7) or *RYR* channels (8) impair Ca^{2+} release from intracellular stores, producing malignant hyperthermia or paralysis (Tables 1, 2). **b**, Comparison of wild-type and mutant (red trace) Na^+ current showing the persistent inward current produced by mutations associated with myotonia and/or periodic paralysis. **c**, Unlike normal muscle (above), action-potential firing in myotonic muscle (below) continues after stimulation has ceased, producing a sustained contraction.

of AChR[13] (see p. 448), the glutamate-binding site of GluR (see p. 456) and the ATP-binding site of Kir6.2 (see p. 470; ref. 28), as well as the cyclic-nucleotide-binding site of CNG channels[9]. This arrangement is energetically more favourable because domain interfaces undergo larger conformational changes on ligand binding. And large conformational changes are needed because the ligand-binding sites lie at some considerable distance from the channel gate (about 50 Å in AChR[13]).

Second, many ligand-gated channels have more than one ligand-binding site, reflecting their multimeric structure. Functionally, this is important as it provides the potential for co-operative interactions. Positive co-operativity is required for the steep ligand dependence of gating that produces a sharp 'on–off' switch of channel activity, as in AChR channels (which have two acetylcholine-binding sites, see p. 448), CNG channels (which have four cyclic-nucleotide-binding sites[9]) and BK channels (which have eight Ca^{2+}-binding sites[8]). In other cases, however, ligand-binding sites do not show co-operative interactions. The four inhibitory ATP-binding sites of the K_{ATP} channel act independently and one molecule of ATP is sufficient to close the channel[27], probably because the channel needs to respond to changes in ATP in a gently graded fashion (see p. 470). As explained in Box 1, the presence of multiple binding sites is important when analysing how heterozygous mutations affect the behaviour of multimeric channels.

A final common feature of ligand-gated channels is that flexible loops (termed gating loops) link the ligand-binding domains to the pore. This is discussed in detail for Cys-loop receptors (see p. 448). Similar loops also link the ligand-binding domains of GluR channels (see p. 456) and the ATP- and Gβγ-binding domain of Kir channels[28,29] (see also p. 470) to the pore.

Accessory proteins

Although ion channels are often viewed as consisting only of pore-forming subunits, this picture is too simplistic. Most channels possess tightly associated accessory subunits, and some form part of much larger macromolecular complexes. Accessory subunits specify the location and abundance of ion channels in the plasma membrane, modulate their biophysical properties and fine-tune their sensitivity to physiological ligands and pharmacological agents. Thus mixing and matching of accessory subunits with different pore-forming subunits contributes to the diversity of ion channels.

Many accessory proteins act like molecular chaperones, facilitating the trafficking of pore-forming subunits to the plasma membrane. In their absence, channels are reduced in number (Ca_V; ref. 30) or totally absent (K_{ATP}; ref. 31). Other ancillary proteins anchor the pore-forming subunit in the membrane or target it to specific locations: rapsyn, for example, clusters AChR at excitatory synapses (see p. 448). Accessory proteins can also be co-opted to serve other functions. The SUR subunit of the K_{ATP} channel has a key role in the metabolic regulation of the channel by hydrolysing ATP[32] and stimulating channel opening (see p. 470); it also confers sensitivity to therapeutic drugs (see p. 470). The MinK and MiRP proteins modulate the single-channel conduct-

Table 1 | Pore-loop channelopathies

Family	Protein	Gene	G/L	Disease and symptoms
Kir	Kir1.1 (ROMK)	*KCNJ1*	L	Bartter's syndrome (renal salt loss)
	Kir2.1 (IRK)	*KCNJ2*	L	Andersen's syndrome
	Kir6.2, K_{ATP} channel α-subunit	*KCNJ11*	L G	Congenital hyperinsulinism Neonatal diabetes; DEND syndrome
	SUR1, β-cell K_{ATP} channel β-subunit	*SUR1*	L	Congenital hyperinsulinism
	SUR2, cardiac K_{ATP} channel β-subunit	*SUR2*	L	Dilated cardiomyopathy
K_V	K_V1.1, neuronal α-subunit	*KCNA1*	L	Episodic ataxia type 1, myokymia, neuromyotonia
	KVLQT1, neuronal α-subunit	*KCNQ1*	L G	Long QT syndrome 1 and possible deafness; Atrial fibrillation; Jervell-Lange-Neilsen syndrome Short QT syndrome
	KCNQ2, neuronal α-subunit	*KCNQ2*	L	Benign neonatal febrile convulsions
	KCNQ3, neuronal α-subunit	*KCNQ3*	L	Benign neonatal febrile convulsions
	KCNQ4, neuronal α-subunit	*KCNQ4*	L	Nonsyndromic deafness
	hERG, I_{Kr} cardiac α-subunit	*KCNH2*	L G	Long QT syndrome 2 Short QT syndrome
	MinK, I_{Ks} cardiac β-subunit	*KCNE1*	L	Long QT syndrome 5 Jervell-Lange-Neilsen syndrome
	MiRP1, I_{Kr} cardiac β-subunit	*KCNE2*	L	Long QT syndrome 6
TRP	TRPP2 (Polycystin 2, PKD2)	*TRPP2*		Autsosomal dominant polycystic kidney disease
	TRPC6	*TRPC6*	G	Focal segmental glomerulosclerosis, defective Mg reabsorption
	TRPML1 (Mucolipin)	*Mcoln1*		Mucolipidosis IV
CNG	Retinal cGMP gated α1-subunit	*CNGA1*	L	Retinitis pigmentosa
	Retinal cGMP gated α3-subunit	*CNGA3*	L	Achromatopsia-2
	Retinal cGMP gated β3-subunit	*CNGB3*	L	Achromatopsia-3
K_{Ca}	BK channel α-subunit	*KCMNA1*	G	Generalized epilepsy with paroxysmal dyskinesia
Na_V	Na_V1.1, neuronal α-subunit	*SCN1A*	G L	Generalized epilepsy with febrile seizures type 2 Severe myoclonic epilepsy of infancy
	Neuronal β-subunit	*SCN1B*	L	Generalized epilepsy with febrile seizures type 1
	Na_V2.1, neuronal α-subunit	*SCN2A*	G	Benign familial neonatal seizures
	Skeletal muscle α-subunit	*SCN4A*	G L	Paramyotonia congenita; Hyperkalemic periodic paralysis; K^+-aggravated myotonia Hypokalemic periodic paralysis
	Cardiac muscle α-subunit	*SCN5A*	G	Long QT syndrome 2; Brugada syndrome; Non-progressive congenital heart block; Progressive cardiac conduction defect
		SCN9A	G	Familial erythermalgia (pain)
Ca_V	Neuronal α-subunit	*CACNA1A*	L G E	Episodic ataxia type 2 Familial hemiplegic migraine Spinocerebellar ataxia type 6
	Retinal α-subunit	*CACNA1F*		Congenital stationary night blindness
	Skeletal muscle α-subunit	*CACNA1S*		Hypokalemic periodic paralysis; malignant hypothermia type 5
	Neuronal β4-subunit	*CACNB4*		Juvenile myoclonic epilepsy; Generalized epilepsy and praxis-induced seizures; Episodic ataxia type 3
	Ca_V1.2, α-subunit	*CACNA1C*		Timothy syndrome

G, gain of function; L, loss of function; E, repeat expansion. The symptom of long and short QT syndrome is ventricular arrhythmia. The symptoms of Jervell-Lange-Neilsen syndrome include deafness and cardiac arrhythmias. Symptoms of autsosomal dominant polycystic kidney disease include cardiac septal defects. Achromatopsia is total colour blindness. The symptoms of Timothy syndrome are multi-organ dysfunction including longQT syndrome and syndactyly. DEND syndrome is characterized by developmental delay, epilepsy, muscle weakness and neonatal diabetes. Anderson's syndrome is a multi-organ disorder that includes potassium-sensitive periodic paralysis and ventricular arrythmia. Brugada syndrome results in ventricular fibrilliation.
For references, see: http://www.ncbi.nlm.nih.gov/entrez/query.fcgi?db=OMIM&cmd=Limits (type in the gene name).

ance, gating and pharmacology of certain kinds of K_V channel (see p. 463), and the inactivation properties of many other voltage-gated channels originate in their accessory subunits[2,30]. Accessory subunits may even serve as kinases that phosphorylate the pore-forming subunit or other proteins[33].

For these reasons, mutations in accessory subunits produce a variety of channelopathies (Tables 1 and 2). For example, mutations in rapsyn decrease AChR density, producing congenital myasthenic syndrome (see p. 448); loss-of-function mutations in SUR cause congenital hyperinsulinaemia by decreasing K_{ATP} channel activity (see p. 470 and ref. 34); and mutations in MinK and MiRP lead to long QT (LQT) syndrome, which can cause fatal arrythmia (see p. 463) (Table 1). Ancillary subunits are also the targets of many therapeutic drugs (see p. 470), making them of key importance to the pharmaceutical industry.

Concepts in channelopathies

The myriad diseases arising from impairment of channel function exemplify the importance of ion channels to the organism (Tables 1 and 2). The range of channelopathies is too great, and too diverse, to cover in a short review — it needs a book[1]. Therefore I shall focus on just a few key concepts or examples that are helpful for understanding the reviews in this Insight. Further information can be found at the Online Mendelian Inheritance in Man website (www.ncbi.nlm.nih.gov/entrez/query.fcgi?db=OMIM&cmd=Limits).

Disorders caused by defective ion-channel function result from several different causes but the term channelopathies is usually confined to disorders resulting from mutations in ion-channel genes themselves (Tables 1 and 2). To date, mutations in over 60 ion-channel genes have been associated with human disease. Inherited channelopathies are usu-

Table 2 | Non-pore-loop channelopathies

Channel protein	Gene	G/L	Disease
Epithelial Na^+ channel (ENaC) β-subunit	*SCNN1B*	G L	Hypertension (Liddle's syndrome) Hypotension (pseudohypoaldosteronism type 1)
Epithelial Na^+ channel (ENaC) γ-subunit	*SCNN1G*	G L	Hypertension (Liddle's syndrome) Hypotension (pseudohypoaldosteronism type 1)
Skeletal muscle sacroplasmic reticulum (SR) calcium release channel	*RYR1*	G G	Malignant hyperthermia (trigged by anaesthetics and muscle relaxants) Central core disease (muscle weakness)
Cardiac SR calcium release channel	*RYR2*	G	Cardiac arrhythmia (catecholaminergic polymorphic VT)
CFTR, epithelial Cl^- channel	*CFTR*	L	Cystic fibrosis
Bestrophin, epithelial Cl^- channel β-subunit	*BEST1*	L	Vitelliform macular dystrophy (Best disease)
ClC1, Cl^- channel skeletal muscle α-subunit	*CLCN1*	L L	Generalized myotonia (Becker's disease) Myotonia congenita (Thomson's disease)
CLC2, Cl^- channel α-subunit	*CLCN2*	L	Several types of epilepsy
ClC5, Cl^- transporter kidney	*CLCN5*	L	Dent's disease (X-linked) and other renal tubular disorders
ClC7, Cl^- transporter	*CLCN7*	L	Osteopetrosis (dense bones), sometimes with blindness
ClC-Ka, Cl^- channel kidney α-subunit	*CLCNKA*	L	Bartter's syndrome
ClC-Kb, Cl^- channel kidney α-subunit	*CLCNKB*	L	Bartter's syndrome
Barttin, Cl^- channel β-subunit		L	Bartter's syndrome with deafness
Cys-loop receptors			
Glycine receptor neuronal α-subunit	*GLRA1*	L	Hyperekplexia (stiff baby syndrome)
$GABA_A$ receptor neuronal α1-subunit	*GABRA1*	L	Juvenile myoclonic epilepsy
AChR skeletal muscle α1-subunit	*CHRNA1*	G	Congenital myasthenia
AChR neuronal α4-subunit	*CHRNA4*	L	Autosomal dominant nocturnal frontal lobe epilepsy
AChR skeletal muscle β1-subunit	*CHRNB1*	G	Congenital myasthenia
AChR skeletal muscle δ-subunit	*CHRND*	G	Congenital myasthenia
AChR skeletal muscle ε-subunit	*CHRNE*	G L	Congenital myasthenia Fast-channel syndrome (muscle weakness)
AChR neuronal β-subunit	*CHRNA1*	L	Autosomal dominant nocturnal frontal lobe epilepsy

G, gain of function. L, loss of function. Generalized myotonia (Becker's disease) and myotonia congenita (Thomson's disease) both result in skeletal muscle hyperexcitability. Bartter's syndrome results in renal salt loss. Congenital myasthenia results in muscle weakness. Hyperekplexia results in muscle contractures when startled. Mutations in ClC5 can cause kidney stones, hypophosphatemic rickets and low molecular weight proteinuria. For references see: http://www.ncbi.nlm.nih.gov/entrez/query.fcgi?db=OMIM&cmd=Limits.

ally very rare. An exception is cystic fibrosis, which affects around 1 in 2,000 people in Northern Europe and the United States. This is a recessive disease, and as many as 1 in 20 people are carriers (see p. 477).

Many channelopathies are genetically heterogeneous, the same clinical phenotype being caused by mutations in different genes. For example, mutations in at least eight different genes give rise to LQT syndrome (Table 1). Furthermore, the same mutation may have greater or lesser effects on different genetic backgrounds. This is one reason why not all carriers of the same disease-causing mutation are affected or why they exhibit different phenotypes. About half the patients carrying the Thr857Met mutation in the Na_V channel α-subunit experience only childhood epilepsy, whereas the other half also have epilepsy as adults[35]. Environmental effects may also influence the extent to which a phenotype is expressed.

Channel-mediated ion flux is determined by the number of channels in the membrane, the fraction of time they remain open (the open probability) and the conductance of the single channel. In principle, alteration of any of these could disrupt channel function, but the last is found only rarely (some CFTR mutations alter single-channel conductance[36]). Loss-of-channel function is commonly the result of mutations that prevent protein synthesis or correct membrane targeting so that the channel density is lower, or that impair channel activity, by disrupting ligand binding, for example. Gain-of-function mutations may also produce disease. Indeed, gain- and loss-of-function mutations in the same gene can produce distinct clinical disorders: neonatal diabetes (due to too little insulin secretion) and congenital hyperinsulinaemia both result from mutations in Kir6.2 (see p. 470; ref. 34).

The form and severity of the clinical phenotype manifested by individuals carrying a given ion-channel mutation depends on several factors. The extent to which the mutation modifies channel function is obviously crucial. However, the relative contribution the channel makes to the electrical activity of the cell is also important. Because cells in different tissues have variable complements of ion channels, their electrical activity can be affected differently. For example, K_{ATP} channels are found in tissues as diverse as brain, muscle and pancreatic beta-cells, but only the most severe mutations produce extra-pancreatic effects[37], because only in beta-cells is the resting potential determined predominantly by the K_{ATP} channel[38].

Different mutations in the same gene may give rise to either recessive or dominant disease, as is the case for ClC1 mutations that cause both myotonia congenita (dominant) and generalized myotonia (recessive)[39]. A dominant disease results if more than 50% of the channel protein is required for normal function (haploinsufficiency) or if the mutant protein interferes with the functional activity of the wild-type protein (the dominant-negative effect), as occurs when a single mutant subunit in a multimeric channel prevents its function. Because heterozygous individuals will express both wild-type and mutant channels in the same cell, heteropolymerization can result in non-functional channels, causing dominant disease. However, a small percentage of homomeric wild-type channels will be present, which may explain why dominant mutations are rarely as severe as recessive ones. Whether or not a mutation is dominant or recessive thus depends on whether the channel is multimeric and if the presence of one (or more) mutant subunit affects the wild-type subunits in the complex. The multimeric nature of ion channels may also influence the severity of recessive disorders (Box 1).

The perils of over- or under-excitement

Ion channels are essential for membrane electrical excitability, and their mutation results in many nerve, muscle and endocrine disorders (Fig. 3). Na_V channels mediate the upstroke of the action potential in

nerve and muscle, first opening in response to depolarization and then rapidly entering a non-conducting inactivated state[2,14] (Fig. 1b). Most mutations in Na_V-channel genes act as gain-of-function mutations[35]. They slow or impair inactivation, which leads to a persistent Na^+ current and membrane hyperexcitability (Fig. 3a, b). In central neurons, such hyperexcitability results in epilepsy[35]; in skeletal muscle it causes myotonia[39] (prolonged muscle contraction); and in the heart it causes LQT syndrome (see p. 463) (Tables 1). It is worth noting that the change in channel activity required to produce disease can be very subtle. A persistent Na^+ current just 2–5% of the maximum is sufficient to cause epilepsy or muscle disorders[41].

Action-potential repolarization is mediated by K_V channels. Unsurprisingly, therefore, loss-of-function mutations in K_V channel subunits prolong the action-potential duration, inducing a hyperexcitability that leads to epilepsy, episodic ataxia and myokymia as well as cardiac disorders (see p. 463; ref. 40; Table 1). Paradoxically, gain-of-function mutations in K^+ channels can also cause cardiac arrhythmia (short QT syndrome; see p. 463) or epilepsy[34,38,41]. The latter may result if the mutation preferentially reduces the excitability of inhibitory neurons[34]. Faster action-potential repolarization may also enable Na_V channels to recover more quickly from inactivation, thereby increasing the firing rate[42].

Epithelial channelopathies

Ion channels are important for ion fluxes across epithelial membranes and thus for salt and water balance. Mutations in the epithelial Na^+ channel (ENaC) lead to enhanced, or reduced, Na^+ uptake in the kidney tubules[42]. Because plasma Na^+ levels influence blood pressure, this causes hereditary hypertension or hypotension, respectively. Mutations in epithelial Cl^- channels[43], or Kir channels[44], also cause renal disorders (Table 2), and cystic fibrosis results from impaired fluid secretion, particularly in the airways (see p. 477). The importance of certain K_V channels in fluid secretion in the cochlear was first recognized from the fact that their mutation causes deafness[40].

Accumulating evidence suggests that ion channels have a role in cell proliferation and cancer. For example, $K_V1.3$ triggers lymphocyte activation and is overexpressed in various tumours, and ectopic expression of the K_V channel Eag1 outside the nervous system triggers tumour formation[45].

Intracellular ion channels

Ion channels are not confined to the plasma membrane. They are found in the membranes of all intracellular organelles, including secretory and endocytotic vesicles, synaptic vesicles, lysozymes, mitochondria and the endoplasmic and sacroplasmic reticulum (SR). Not surprisingly, mutations in their genes also cause disease. Thus, mutations in the SR ryanodine receptor, which regulates Ca^{2+} release from intracellular stores, lead to malignant hyperthermia, a disorder in which common inhalation anaesthetics produce uncontrolled muscle contractions and a potentially fatal rise in body temperature[46]. The eukaryotic cousins of the Cl^- transporters described by Miller in this issue (p. 484) are crucial for acidification of endocytotic vesicles in epithelial tissues[43]. Their mutation can produce osteopetrosis, in which the bone marrow is replaced by bone, or Dent's disease, which is characterized by low-molecular-weight proteinuria, kidney stones, hypercalciuria, nephrocalcinosis and occasionally renal failure[43].

Other types of channel disease

Although not strictly considered as channelopathies, mutations in other genes can adversely affect ion-channel function. These include genes involved in trafficking, localizing and anchoring channels in the plasma membrane or those that influence the concentration of ligands or modulators of channel function. Congenital myasthenia, for example, can be produced by mutations in rapsyn, by a deficiency of acetylcholinesterase or acetylcholine production, as well as by mutations in the AChR itself (see p. 448). Similarly, mutations in regulatory genes can cause diabetes[37] by affecting the activity of ion channels in pancreatic beta-cells. Autoantibodies against ion channels also cause a range of disorders, including myasthenia gravis[47], Lambert–Eaton myasthenic syndrome[47], acquired neuromyotonia[47,48] and CNS disorders[48]. These are sometimes known as the autoimmune channelopathies. Ion channels are also the target of a large and diverse group of toxins that bind to ion channels and either inhibit or enhance their activity[14]. In addition, therapeutic drugs can cause iatrogenic disease if they interact adversely with ion channels. These are sometimes referred to as acquired channelopathies. The best-known example is that of drugs that inhibit the cardiac K_V channel hERG and thereby produce acquired LQT syndrome (see p. 463). Interestingly, specific polymorphisms (gene variants) seem to predispose certain individuals to drug-induced LQT[49].

Future perspectives

Almost 350 years ago, William Harvey wrote: "Nor is there any better way to advance the proper practice of medicine than … by careful investigation of the rarer forms of disease". Studies of channelopathies illustrate this maxim's current relevance, for they have identified novel ion-channel genes, illuminated relationships between channel structure and function, and revealed diverse and unexpected physiological roles for ion channels. And, importantly, they have also led to new therapeutic practice, to new diagnostic tests for genetic disease, to routine screening of drugs to prevent acquired channel disorders and to novel drug therapy.

Ion-channel research is now poised at an exciting point. In the next decade, we anticipate new high-resolution structures and rapid advances in our understanding of how ion channels work. With luck, we will discover the key structural elements that distinguish ion channels from their cousins the transporters. A better understanding of how gating works, how accessory subunits regulate channel structure and how lipids interact with ion channels is likely. Advances are also expected from studies of channelopathies, both in terms of structure–function relationships and of how channels contribute to cellular and systems physiology. A particular challenge will be to elucidate the extent to which polymorphisms in ion-channel genes predispose individuals towards common polygenic diseases such as type 2 diabetes, hypertension, obesity and cardiac disease. At least in the case of diabetes[37] and hypertension[50] there is evidence that this is the case. We can also expect that advances in ion-channel research will lead to new therapies, in the form of new drugs and of drugs tailored to the individual's genetic profile. ■

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