



Ion channels: Function unravelled by dysfunction

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Ion channels allow the passage of specific ions and electrical charge. Plasma membrane channels are, for example, important for electrical excitability and transepithelial transport, whereas intracellular channels have roles in acidifying endosomes or in releasing Ca^{2+} from stores. The function of several channels emerged from mutations in humans or mice. The resulting phenotypes include kidney stones resulting from impaired endocytosis, hypertension, defective insulin secretion, cardiac arrhythmias, neurological diseases like epilepsy or deafness and even 'developmental' defects such as osteopetrosis.

Ion channels are integral membrane proteins that form a pore to allow the passage of specific ions by passive diffusion. Most, if not all, ion channels undergo conformational changes from closed to open states, and once open, channels allow the passage of thousands of ions. This distinguishes them from transporters and pumps, which can also transport ions but only one (or a few) at a time. The opening and closing of channels can be controlled by various means, including voltage, the binding of ligands such as intracellular Ca^{2+} or extracellular neurotransmitters, and post-translational modifications such as phosphorylation. Further levels of control are provided by the insertion of the channel into the target membrane, and by its regulated retrieval and degradation. Many ion channels are protein complexes of identical or homologous subunits that participate in forming the pore. These often associate with other channel-specific subunits that are structurally unrelated and may regulate the channels.

There is an astounding molecular variety of ion channels; for example, there are more than 60 different genes that encode K^+ channels. It is a major challenge to understand why organisms need such a plethora of molecules with the same basic function of conducting ions. The elucidation of many human ion-channel diseases, 'channelopathies', and the disruption of genes that encode ion channels in mice provide fascinating insights into the diverse roles of ion channels. Rather than giving an encyclopaedic summary of channelopathies (Table 1)^{1,2}, we will discuss enlightening examples of ion-channel diseases that illustrate two major roles of plasma membrane channels, transepithelial transport and the control of electrical excitability, and examples that reveal the function of intracellular channels in the acidification of endosomes, with secondary effects on endocytosis.

Plasma membrane channels in transepithelial transport

Ions and water (which follows osmotically) are transported across epithelial barriers in the gastrointestinal tract, the kidney, glands and many other organs. Vectorial ion transport across epithelia is a consequence

of the selective expression of ion channels and transporters in apical or basolateral membranes. Typically, the ion is transported across one of these membrane domains in an active transport process: primary active transport occurs through transport ATPases (for example, the $(\text{Na}^+ + \text{K}^+)\text{ATPase}$), and secondary active transport couples transmembrane ion gradients established by primary active transport (for example, the Na^+ gradient) to the transport of another ion (for example, Cl^- in $\text{Na}-\text{Cl}$ cotransporters). This active transport raises (or lowers) the cytosolic concentration of the ion above (or below) its electrochemical equilibrium. The presence of an appropriate ion channel in the opposing membrane then leads to a purely passive ion efflux or influx, respectively. The combination of these transport processes results in net transport across the epithelial layer.

Probably the best known human disease caused by defective transepithelial transport is cystic fibrosis (CF). In CF, the production of a thick, viscous mucus in the lung, in combination with bacterial infections, progressively causes severe pulmonary dysfunction. The epithelial transport defect in CF also leads to pathological changes in other epithelial organs, most prominently the pancreas and the intestine.

Even fifteen years after the identification of the gene underlying this disease³ — CFTR, for 'cystic fibrosis transmembrane conductance regulator' — many details of CF pathology are poorly understood or controversial. CFTR, although belonging to the ABC-transporter family that generally use ATP to 'pump' diverse substrates, functions as a cAMP-activated Cl^- channel that is present in apical membranes of several epithelia. In the intestine, for instance, CFTR has a role in Cl^- secretion. Cholera results from an inappropriate activation of this channel by cAMP, the production of which is stimulated by cholera toxin. Conversely, loss of CFTR leads to thick faeces in some infants with CF and in CFTR-knockout mice.

Mouse models where CFTR is mutated or knocked out do not, however, mimic the most dangerous human CF pathology; that is, lung disease with bacterial infection. CFTR is important for salt transport across pulmonary epithelia, and susceptibility to bacterial infections has been attributed to the salt sensitivity of antibacterial peptides that are secreted into the fluid covering the lung epithelia^{4,5}. Moreover, there may be differences between the glycosylation of secreted mucins of disease CFTR

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Table 1 Ion channels associated with human disease

Protein	Gene	Subunit	Ligand	Disease	Functional defect
Cation channels					
CHRNA1	<i>CHRNA1</i>	α	ACh	myasthenia congenita	motor endplate
CHRNA4	<i>CHRNA4</i>	α	ACh	autosomal-dominant nocturnal frontal lobe epilepsy	hyperexcitability
CHRN2	<i>CHRN2</i>	β	ACh	autosomal-dominant nocturnal frontal lobe epilepsy	hyperexcitability
CHRNE	<i>CHRNE</i>	ϵ	ACh	congenital myasthenic syndrome	motor endplate
Polycystin-2	<i>PKD2</i>	α		autosomal-dominant polycystic kidney disease (ADPKD)	
CNGA3	<i>CNGA3</i>	α	cGMP	achromatopsia	visual signal transduction
CNGB1	<i>CNGB1</i>	β	cGMP	retinitis pigmentosa	visual signal transduction
CNGB3	<i>CNGB3</i>	β	cGMP	achromatopsia	visual signal transduction
Sodium channels					
Na _v 1.1	<i>SCN1A</i>	α		generalised epilepsy with febrile seizures (GEFS+)	hyperexcitability
Na _v 1.2	<i>SCN2A</i>	α		generalised epilepsy with febrile & afebrile seizures	hyperexcitability
Na _v 1.4	<i>SCN4A</i>	α		paramyotonia congenita, potassium-aggravated myotonia hyperkalaemic periodic paralysis	hyperexcitability
Na _v 1.5	<i>SCN5A</i>	α		long-QT syndrome/Brugada syndrome	heart action potential
SCN1B	<i>SCN1B</i>	β		generalised epilepsy with febrile seizures	hyperexcitability
ENaC α	<i>SCNN1A</i>	α		pseudohypoaldosteronism type 1	renal salt loss
ENaC β	<i>SCNEB</i>	β		pseudohypoaldosteronism type 1 pseudoaldosteronism (Liddle syndrome)	renal salt loss increased renal Na ⁺ uptake
ENaC γ	<i>SCNN1G</i>	γ		pseudohypoaldosteronism type 1 pseudoaldosteronism	renal salt loss increased renal Na ⁺ uptake
TRPM6	<i>TRPM6</i>	α		hypomagnesaemia	renal Mg ²⁺ loss
Potassium channels					
K _v 1.1	<i>KCNA1</i>	α		episodic ataxia with myokymia	hyperexcitability
KCNQ1	<i>KCNQ1</i>	α		autosomal-dominant long-QT syndrome with deafness autosomal-recessive long-QT syndrome	heart action potential/ inner ear K ⁺ secretion heart action potential
KCNQ2	<i>KCNQ2</i>	α		Benign familial neonatal convulsions (BFNC), also with myokymia	hyperexcitability
KCNQ3	<i>KCNQ3</i>	α		Benign familial neonatal convulsions	hyperexcitability
KCNQ4	<i>KCNQ4</i>	α		autosomal-dominant deafness	inner ear K ⁺ recycling
KCNH2	<i>KCNH2</i>	α		long-QT syndrome	heart action potential
Kir1.1/ ROMK	<i>KCNJ1</i>	α		Bartter syndrome	renal salt loss
Kir2.1	<i>KCNJ2</i>	α		long-QT syndrome with dysmorphic features	heart action potential
Kir6.2	<i>KCNJ11</i>	α		persistant hyperinsulinaemic hypoglycaemia of infancy diabetes mellitus	insulin hypersecretion insulin hyposecretion
SUR1	<i>SUR1</i>	α		persistant hyperinsulinaemic hypoglycaemia of infancy	insulin hypersecretion
SUR2	<i>SUR2</i>	α		dilated cardiomyopathy	metabolic signalling
KCNE1	<i>KCNE1</i>	β		autosomal-dominant long-QT syndrome with deafness autosomal-recessive long-QT syndrome	heart action potential heart action potential
KCNE2	<i>KCNE2</i>	β		long-QT syndrome	heart action potential
KCNE3	<i>KCNE3</i>	β		hypokalaemic periodic paralysis (?)	
Calcium channels					
Ca _v 1.1	<i>CACNA1S</i>	α		malignant hyperthermia, periodic paralysis	muscle Ca ²⁺ homeostasis
Ca _v 1.2	<i>CACNA1C</i>	α		Thymothy syndrome	multisystem disorder
Ca _v 1.4	<i>CACNA1F</i>	α		X-linked congenital stationary night blindness	visual signal transduction
Ca _v 2.1	<i>CACNA1A</i>	α		familial hemiplegic migraine/episodic ataxia/ spinocerebellar ataxia type 6	
EFHC1	<i>EFHC1</i>	β		juvenile myoclonus epilepsy	
RyR1	<i>RYR1</i>	α		malignant hyperthermia, central core disease	muscle Ca ²⁺ homeostasis
RyR2	<i>RYR2</i>	α		arrhythmogenic right ventricular dysplasia type 2	cardiac Ca ²⁺ homeostasis

Table 1 Ion channels associated with human disease

Protein	Gene	Subunit	Ligand	Disease	Functional defect
Chloride channels					
CFTR	<i>ABCC7</i>	α		cystic fibrosis	epithelial transport defect
CIC-1	<i>CLCN1</i>	α		myotonia (autosomal-recessive or -dominant)	defective muscle repolarization
CIC-2	<i>CLCN2</i>	α		epilepsy	?
CIC-5	<i>CLCN5</i>	α		Dent's disease	defective endosome acidification
CIC-7	<i>CLCN7</i>	α		osteopetrosis (recessive or dominant)	defective bone resorption
CIC-Kb	<i>CLCNKB</i>	α		Bartter syndrome type III	renal salt loss
Barttin	<i>BSND</i>	β		Bartter syndrome type IV with deafness	renal salt loss/endolymph secretion
Bestrophin	<i>VMD2</i>			vitelliform macular dystrophy	?
GLRA1	<i>GLRA1</i>	α	glycine	hyperekplexia	impaired synaptic inhibition
GABA α 1	<i>GABRA1</i>	α	GABA	juvenile myoclonus epilepsy	impaired synaptic inhibition
GABA γ 2	<i>GABRG2</i>	γ	GABA	epilepsy	impaired synaptic inhibition
Water channels					
MIP26	<i>AQP0</i>			cataract	cell adhesion (?)
AQP2	<i>AQP2</i>			diabetes insipidus	renal water loss
Gap junctions					
Cx26	<i>GJB2</i>			deafness (autosomal-recessive and -dominant)	inner ear K ⁺ recycling
Cx30	<i>GJB4</i>			autosomal-dominant deafness	inner ear K ⁺ recycling
Cx31	<i>GJB3</i>			autosomal-dominant deafness	inner ear K ⁺ recycling
Cx32	<i>GJB1</i>			polyneuropathy (CMT)	
Cx46.6	<i>GJA12</i>			Pelizaeus-Merzbacher-like disease	

variants and normal ones due to an altered pH in the secretory pathway⁶. In addition, CFTR is itself a receptor for certain bacteria⁷.

The situation is further complicated by the large number of suggested regulatory roles for CFTR. CFTR has been proposed to regulate the epithelial Na⁺ channel ENaC^{8–10}, Cl[−] channels distinct from CFTR¹¹ and the ROMK K⁺ channel¹², among others. Some of these proposed regulatory relationships may be clinically important. Whereas CFTR-null mice have no obvious lung pathology, mice overexpressing ENaC (which is mostly thought to be downregulated by CFTR⁸) in airways exhibited a lung phenotype characterized by its strange sound¹³. Intriguingly, the electrogenic anion exchangers SLC26A3 and SLC26A6 may be directly activated by CFTR¹⁴. Certain CFTR mutations impair the activation of chloride–bicarbonate exchange through SLC26A3 and SLC26A6 (ref. 14), thereby resulting in decreased pancreatic HCO₃[−] secretion, which occurs commonly in CF.

Channelopathies of the kidney have better-understood pathological mechanisms. After the primary urine is formed by glomerular filtration, ions, water and organic substances are reabsorbed by tubular epithelial cells. Nephron segments are equipped with different sets of channels and transporters to perform specialized tasks. Although the bulk of solutes and water are reabsorbed in the proximal tubule and the loop of Henle, the distal nephron is involved in the fine tuning of Na⁺, K⁺ and acid excretion, regulated by hormones such as aldosterone and antidiuretic hormone.

A key factor in controlling Na⁺ reabsorption and in regulating blood pressure is ENaC, which is present in apical membranes of the distal nephron (Fig. 1c). Given the inwardly directed Na⁺ gradient, ENaC mediates the passive Na⁺ influx from the urine into the cell. Na⁺ is then pumped out into the blood over the basolateral membrane by the

(Na⁺ + K⁺)ATPase, resulting in Na⁺ reabsorption from the urine. Gain-of-function mutations in ENaC lead to Liddle syndrome, a dominant form of hypertension. These mutations delete or change a proline-rich, tyrosine-containing motif (PY motif) in the cytosolic carboxyl terminus of the protein. This motif interacts with the ubiquitin ligase, Nedd4 (refs 15, 16). Normally, ubiquitination of ENaC by this enzyme enhances the endocytosis and degradation of the channel. Hence, mutations in Liddle disease increase the plasma membrane expression of ENaC by impairing its ubiquitination. The predicted increase in Na⁺ reabsorption leads to high blood pressure, which agrees with the known correlation between high salt intake and hypertension.

The stimulation of Na⁺ reabsorption by aldosterone could be due in part to a phosphorylation of Nedd4 by Sgk1 kinase¹⁷, which impairs the interaction between Nedd4 and ENaC, thereby increasing ENaC plasma membrane expression. This kinase is induced by glucocorticoids and mineralocorticoids (for example, aldosterone), and undoubtedly has multiple roles. The disruption of Sgk1 in mice, however, only slightly affects Na⁺ reabsorption¹⁸, possibly suggesting redundant mechanisms of Nedd4 phosphorylation.

Whereas gain-of-function mutations in ENaC underlie hypertension, loss-of-function mutations cause recessive pseudohypoaldosteronism type I (PHA1)¹⁹. In this syndrome, renal salt loss leads to a secondary increase in aldosterone. This, however, cannot normalize renal Na⁺ reabsorption because ENaC, one of its downstream targets, is non-functional.

Pseudohypoaldosteronism type II (PHA2), which is associated with high blood potassium and hypertension that probably results from increased renal Na⁺ absorption, is caused by mutations in two genes encoding the protein kinases, WNK1 and WNK4 (ref. 20). Both kinases

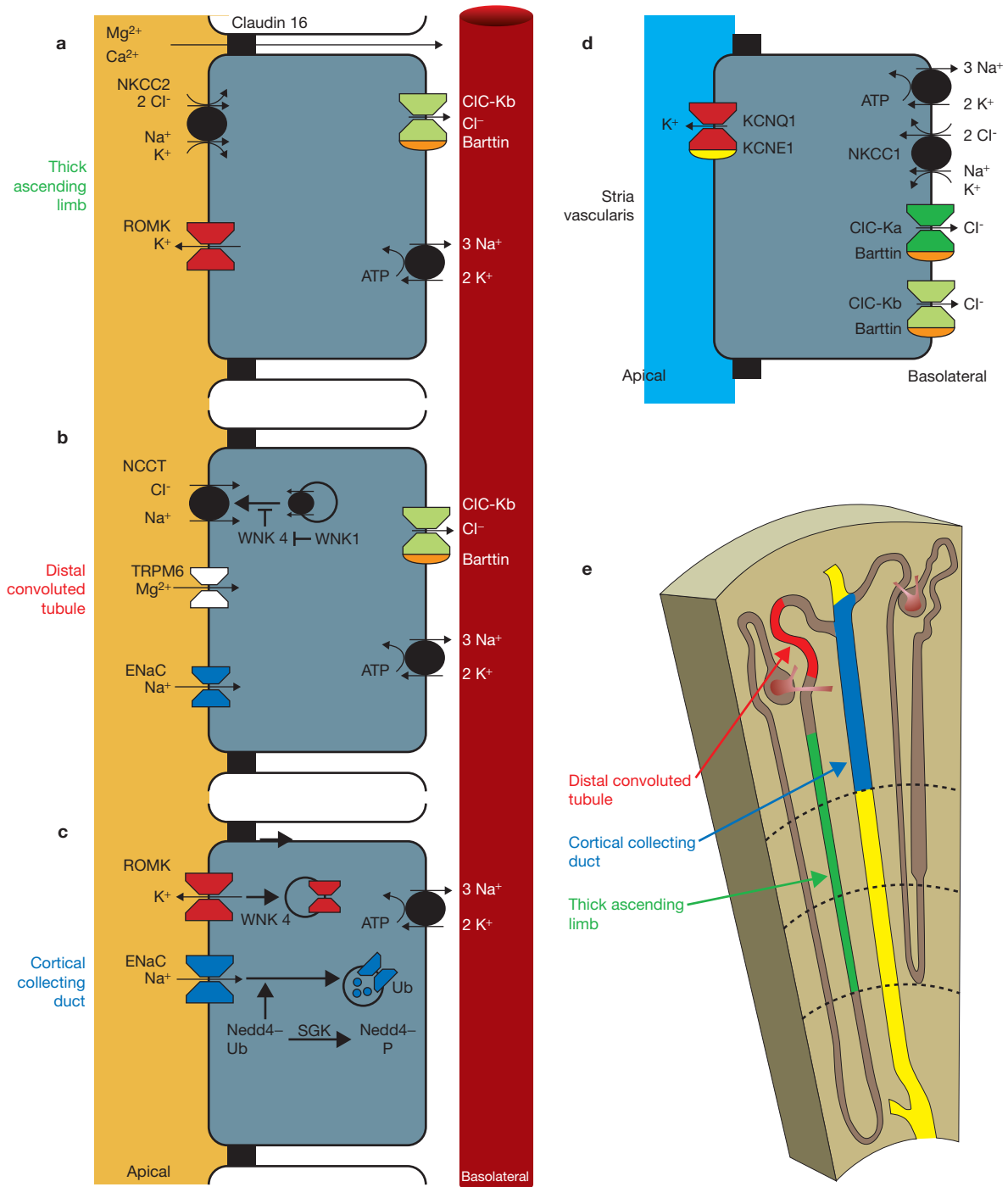


Figure 1 Ion transport processes in the kidney and the stria vascularis, the endolymph-secreting epithelium of the inner ear. **(a)** In cells of the thick ascending limb (TAL) of Henle's loop, apical NKCC2 co-transporters drive Cl⁻ uptake. The K⁺ channel ROMK is indispensable for apical K⁺ recycling. Cl⁻ exits through basolateral channels formed by ClC-Kb and barttin. Mutations in all four genes can cause Bartter syndrome. In the TAL Mg²⁺ is reabsorbed paracellularly in a pathway that involves claudin 16, whereas in the distal convoluted tubule (DCT) Mg²⁺ is transported transcellularly by TRPM6. Mutations in either gene can result in renal Mg²⁺ loss. **(b)** Salt uptake in the DCT is mediated by NCCT. An increase of NaCl uptake through NCCT contributes to pseudohypoaldosteronism (PHA) type II, which is caused by mutations of WNK1 or WNK4. Both kinases differentially affect the apical

insertion of NCCT NaCl cotransporters. In the cortical collecting duct, WNK4 stimulates the endocytosis of ROMK. PHAI-specific WNK4 mutations further increase this effect. The predicted decrease of ROMK in the apical membrane leads to decreased K⁺ secretion. **(c)** Na⁺ absorption in the CCD is mediated by the epithelial sodium channel ENaC. Its loss of function causes PHAI, whereas gain-of-function mutations underlie hereditary hypertension. **(d)** In marginal cells of the stria vascularis, the intracellular K⁺ concentration is raised by basolateral NKCC1 in conjunction with the (Na⁺+K⁺)ATPase. K⁺ is secreted into the endolymph through KCNQ1/KCNE1 K⁺ channels. Parallel basolateral ClC-Ka/barttin and ClC-Kb/barttin channels recycle Cl⁻ and mutations in these channels are associated with deafness. **(e)** The nephron, colour-coded as indicated in **a-c**.

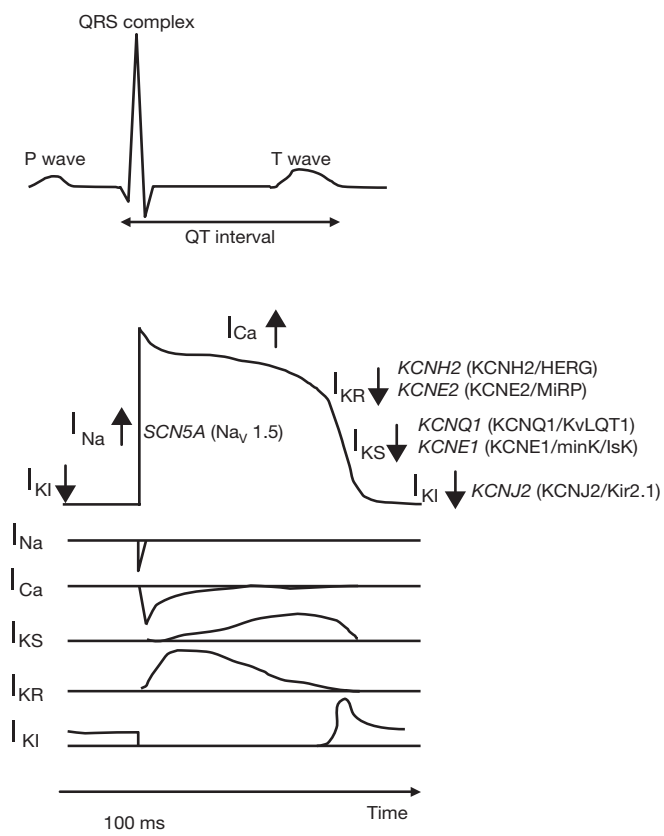


Figure 2 Relationship of the electrocardiogram (ECG), the cardiac action potential and the different currents contributing to the cardiac action potential. The names of the corresponding genes that are mutated in long-QT syndrome are shown, with the encoded channel protein in brackets. The P wave of the ECG corresponds to propagation of excitation in the atria, the QRS complex is a consequence of the propagation of excitation in the ventricles, whereas the repolarization of the ventricle can be seen as the T wave.

are expressed in the distal nephron as part of a signalling pathway that includes the Na–Cl cotransporter NCCT and the K^+ channel ROMK (also known as Kir1.1) as downstream targets. WNK4 co-expression inhibits the trafficking of NCCT to the plasma membrane^{21,22}, whereas WNK1 prevents WNK4-mediated inhibition of NCCT²² (Fig. 1b). Mutations of WNK4 that are found in individuals with PHAII abolish the inhibition of NCCT trafficking, implying a pathologically increased NaCl absorption in the connecting segment, the site of NCCT expression. Interestingly, wild-type WNK4 also reduced the plasma membrane expression of ROMK. In contrast to the mechanism proposed for NCCT, however, this reduced expression of ROMK involved a stimulation of endocytosis²³ (Fig. 1c). Notably, the specific WNK4 mutations found in PHAII further stimulated retrieval of ROMK from the plasma membrane in a gain-of-function mechanism²³. The predicted decrease of ROMK in the apical membrane *in vivo* should lead to decreased renal K^+ secretion and hence to the increased serum potassium that is observed in PHAII.

Whereas a reduced plasma membrane expression of ROMK in the distal nephron contributes to PHAII²³, its total loss indirectly impairs NaCl reabsorption in the thick ascending limb of Henle's loop, a nephron segment that is highly active in NaCl reabsorption. This leads to Bartter syndrome, a genetically heterogeneous disease characterized by severe renal salt loss. Individuals with Bartter syndrome can also have

mutations in NKCC2, an apical NaK2Cl cotransporter²⁴, in CIC-Kb²⁵, a basolateral Cl^- -channel α -subunit, and in barttin²⁶, a β -subunit of CIC-K channels that is required for their transport to the plasma membrane²⁷. These findings beautifully support a transport model for this nephron segment (Fig. 1a): powered by the Na^+ gradient created by the basolateral ($Na^+ + K^+$)ATPase, Na^+ , K^+ and Cl^- are taken up from the urine across the apical membrane by NKCC2. K^+ ions taken up by NKCC2 must be recycled over the apical membrane, a job performed by ROMK; Na^+ is pumped out by the basolateral ($Na^+ + K^+$)ATPase; and Cl^- diffuses passively through basolateral CIC-Kb/barttin Cl^- channels.

In addition to causing renal salt wasting, loss-of-function of the Cl^- -channel β subunit barttin causes deafness²⁶, which probably results from an impaired secretion of K^+ into the scala media of the inner ear. A high K^+ concentration in this compartment is crucial for hearing, as it allows an influx of K^+ through mechanosensitive channels of sensory hair cells. The transport model for the epithelium that generates this high concentration of K^+ , the stria vascularis (Fig. 1d), is derived mostly from the phenotypes observed in channelopathies and mouse models. K^+ is taken up across the basolateral membrane of marginal cells by the ($Na^+ + K^+$)ATPase and basolateral NKCC1 NaK2Cl cotransporters. K^+ ions are then secreted through apical K^+ channels that are composed of KCNQ1 pore-forming α -subunits and KCNE1 β -subunits. The recessive Jervell and Lange-Nielsen syndrome, which combines cardiac arrhythmia with deafness, is associated with mutations in the genes encoding either of these subunits^{28,29}. Interestingly, mutations in the related KCNQ4 channel, which is expressed in hair cells, also cause deafness³⁰. Analogous to the role of ROMK in apical K^+ recycling in the thick ascending limb of Henle's loop (Fig. 1a), CIC-Ka/barttin and CIC-Kb/barttin recycle Cl^- over the basolateral membrane of marginal cells of the stria vascularis²⁷ (Fig. 1d). As that membrane expresses both CIC-Ka and CIC-Kb, mutations in CIC-Kb cause just renal salt loss²⁵, whereas the loss of the barttin subunit, which is common to the two channels, additionally causes deafness. This model²⁷ was confirmed by the identification of a family carrying homozygous mutations in both CIC-Ka and CIC-Kb³¹.

Depending on the particular epithelium, a significant proportion of transepithelial transport occurs through the clefts between epithelial cells (that is, paracellularly). This was impressively demonstrated by the finding that renal loss of Mg^{2+} is associated with mutations not only in the cation channel TRPM6 (ref. 32), but also in claudin16 (paracellin), a tight-junction protein³³. This finding reveals that extracellular pathways between epithelial cells can be surprisingly ion-selective. Indeed, changes of paracellular ion selectivity are observed after overexpression of claudin isoforms³⁴. The importance of paracellular pathways was further strengthened by the recent finding that the kinase WNK4 — mentioned above for its role in hypertension — stimulates paracellular Cl^- permeability³⁵, in addition to its effects on Na–Cl cotransport and a K^+ channel.

Plasma membrane channels and electrical excitability

Ion channels are important to excitable cells such as neurons, cardiac and skeletal muscle. Unsurprisingly, several channelopathies affect those tissues. Action potentials — the basic 'all-or-none' pattern of electrical excitation of nerve and muscle — are initiated by the opening of voltage-dependent Na^+ channels. This leads to a depolarizing influx of Na^+ , which is stopped by an intrinsic inactivation of Na^+ channels, and the membrane voltage is repolarized to its normal value by K^+ efflux through K^+ channels, and sometimes also by an influx of Cl^- (especially in skeletal muscle). This suggests that electrical hyperexcitability might result from either gain-of-function mutations in Na^+ channels, or loss-of-function mutations in K^+ (or Cl^-) channels. This simple prediction is indeed fulfilled in many channelopathies, including cardiac arrhythmias^{36–40} and

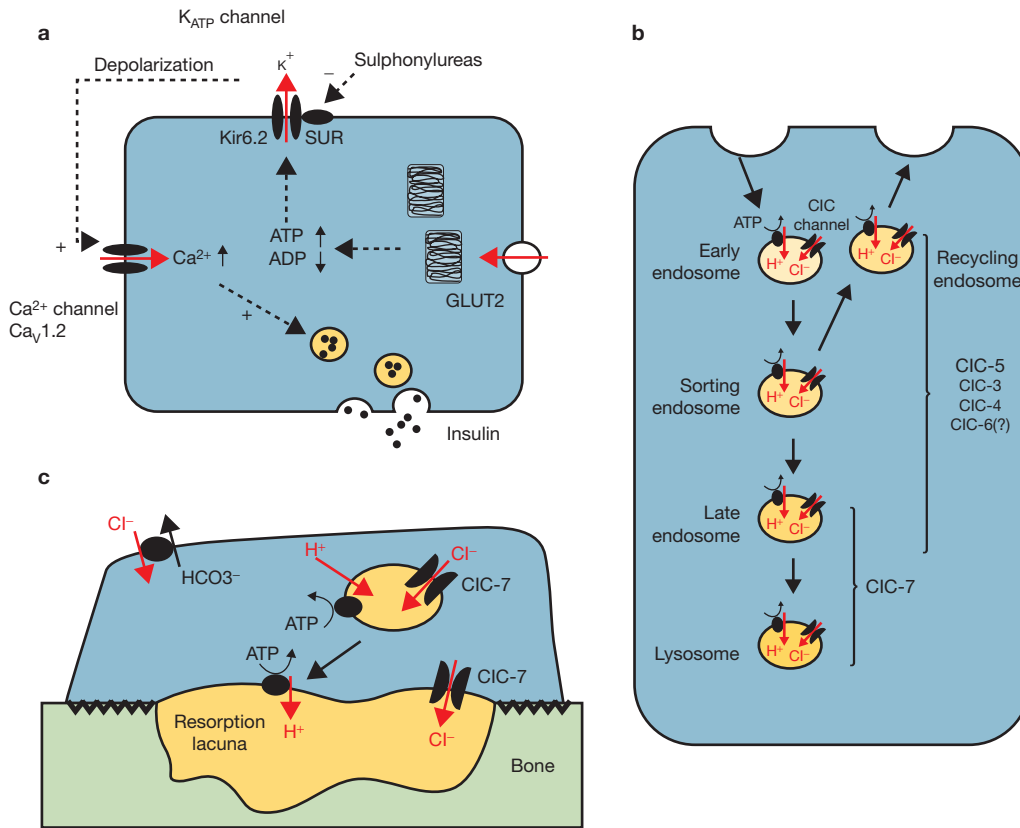


Figure 3 Ion channels in endocytosis and exocytosis. **(a)** Excitation–secretion coupling of the pancreatic β -cell. Glucose, taken up by GLUT2, is metabolized. The ensuing rise of cytoplasmic ATP inhibits K_{ATP} , a heteromer of Kir6.2 and SUR subunits, which determines most of the resting potential of β -cells. This inhibition enables inward currents to depolarize the β -cell, which in turn opens voltage-dependent Ca^{2+} channels. The ensuing increase of intracellular Ca^{2+} causes the exocytosis of insulin-containing vesicles. **(b)** Model for the acidification of the endosomal/lysosomal pathway. Vesicles of the endosomal/lysosomal pathway are acidified by V-type H^+ -ATPases.

The voltage over the vesicular membrane that would be generated by this process is neutralized by chloride entering the vesicles through channels of the CLC type; for example, CIC-5 in endosomes and CIC-7 in lysosomes. The localization to specific compartments, however, is less clear for CIC-3, -4 and -6. **(c)** In bone-resorbing osteoclasts, proton pumps and CIC-7 Cl^- channels are inserted into the membrane facing the bone surface. Thereby the resorption lacuna obtains its characteristic acidic pH, which is essential for the dissolution of the mineral phase as well as the enzymatic degradation of the organic bone matrix.

myotonic syndromes of skeletal muscle^{41–43}. Given the complexity of the CNS, where hyperexcitability of inhibitory neurons could lead to a dampening of downstream neuronal circuits, it is surprising that this simplistic view apparently holds true for some forms of epilepsy^{44–48}.

The duration of the cardiac action potential is very long (Fig. 2), because the Ca^{2+} influx through voltage-sensitive Ca^{2+} channels during its plateau phase is needed for the contraction of the cardiac muscle. Mutations that prolong the action potential, which is reflected in the Q–T interval in electrocardiograms, indirectly cause cardiac arrhythmias in the so-called ‘long-QT’ syndrome (LQTS). The slow repolarization of the cardiac action potential is mainly due to the KCNQ1 K^+ channel, which activates very slowly after depolarization when associated with KCNE1 β -subunits. Dominant-negative mutations in either subunit can lead to a dominant form of LQTS by impairing the repolarization of the cardiomyocyte. A total loss of function (with mutations on both alleles) leads to the recessive Jervell and Lange–Nielsen syndrome^{28,29}. Mutations in another K^+ channel, HERG (KCNH2), can also underlie LQTS³⁹, and mutations in KCNE2, that can associate with HERG, are also linked with this syndrome⁴⁰. Mutations in a further K^+ channel, KCNJ2 (also known as Kir2.1), lead to a form of LQTS that is associated with dysmorphic features and epilepsy (Andersen syndrome)⁴⁹.

Mutations in the cardiac Na^+ -channel gene *SCN5A* can underlie different cardiac pathologies, including LQTS³⁶. Whereas LQTS-associated mutations in K^+ channels generally entail a loss of channel function, those in *SCN5A* generally impair channel inactivation^{50,51}. This leads to additional, late Na^+ currents that increase cardiac excitability and action potential length.

Mutations affecting Na^+ channel inactivation were also identified in the skeletal muscle isoform *SCN4A* (refs 52, 53). They are associated with hyperexcitability as in paramyotonia^{52,54}, or with periodic paralysis precipitated by high⁴³ or low⁵⁵ serum concentrations of K^+ (hyper- and hypokalaemia, respectively). Paralysis might result from the voltage-dependent inactivation of Na^+ channels that is elicited by a moderate membrane depolarization induced by a subpopulation of non-inactivating (mutant) Na^+ channels. Besides *SCN4A* (ref. 55), the major gene underlying hypokalaemic periodic paralysis is *CACNL1A3* (refs 56, 57), which encodes the skeletal muscle Ca^{2+} -channel isoform. An amino-acid exchange⁵⁸ in the K^+ -channel β -subunit KCNE3 (ref. 59) was also reported to cause hypokalaemic periodic paralysis⁵⁸, but it is probably a benign polymorphism^{60,61}. Myotonia, an impairment of muscle relaxation, is a consequence of trains of action potentials caused by electrically hyperexcitable muscle membranes. Whereas temperature-dependent paramyotonia is caused by mutations in *SCN4A* (refs 52, 54),

recessive and dominant 'pure' myotonia is caused by mutations in the Cl⁻ channel *CLC-1* (refs 41, 62, 63), which contributes to action potential repolarization in skeletal muscle.

Channelopathies in the CNS include forms of epilepsy^{44–48,64}, ataxia⁶⁵ and migraine⁶⁶. In an already familiar pattern, mutations that impair inactivation of brain Na⁺ channels lead to various forms of epilepsy^{48,67}. Mutations were identified in the ion-conducting α -subunit *SCN1A* (ref. 46) and its modulatory β -subunit *SCN1B* (ref. 47), as well as in the *SCN2A* α -subunit^{48,64}. Mutations causing rare forms of epilepsy are also found in several K⁺-channel subunits, notably in *KCNQ2* and *KCNQ3* (refs 44, 45). These subunits can form heteromeric channels^{68,69} that show properties of the so-called 'M-current'⁶⁹. The role of this current in sensitively regulating neuronal excitability may explain that already a slight loss of *KCNQ2/3* current suffices to cause epilepsy⁶⁸.

Chloride has important inhibitory roles in the CNS. The hyperpolarizing Cl⁻ influx through ligand-gated GABA_A- and glycine-receptor channels dampens post-synaptic neuronal activity. It comes as no surprise that loss-of-function mutations in two GABA_A-receptor subunits (*GABRA1* and *GABRG2*) were identified in individuals with certain forms of epilepsy^{70–72}. Loss-of-function mutations in glycine receptors, which have prominent roles in the spinal cord, lead to startle disease, which is characterized by exaggerated reflexes in response to sudden shocks⁷³. Although mouse models indicate⁷⁴ that the K–Cl cotransporter *KCC2* is important for establishing the low intraneuronal Cl⁻ concentration that is crucial for GABAergic and glycinergic synaptic inhibition, no mutation in the equivalent human gene is known. A similar role to *KCC2* has been ascribed to the voltage-gated Cl⁻ channel *CLC-2*. Whereas *CLC-2*-knockout mice do not exhibit epilepsy⁷⁵, *CLC-2* mutations are associated with generalized epilepsy in some families⁷⁶. However, key aspects of the functional analysis of the mutations⁷⁶ could not be reproduced⁷⁷.

A fascinating example of metabolically controlled excitation–secretion coupling is given by pancreatic β -cells (Fig. 3a). These cells sense plasma levels of glucose and convert this information into insulin secretion, resulting in a negative feedback loop as insulin lowers systemic glucose levels. Glucose is taken up across the plasma membrane of β -cells by *Glut2*, and its metabolism increases cellular ATP levels. Cytoplasmic ATP directly inhibits *K_{ATP}* — a K⁺ channel that determines most of the resting voltage of β -cells. When this inhibition is strong enough, the cell is depolarized by small inward currents through other channels. The depolarization activates voltage-dependent Ca²⁺ channels and the rise in cytoplasmic Ca²⁺ triggers the exocytosis of insulin-containing vesicles. Human mutations in the subunits of *K_{ATP}* lead to pathologically enhanced or reduced insulin secretion^{78–80}.

K_{ATP} is an octamer of four ion-conducting Kir6.2 α -subunits and four SUR1 (sulfonylurea receptor 1) β -subunits. Whereas Kir6.2 subunits carry the inhibitory ATP-binding site, SUR1 confers sensitivity to sulphonylureas. These drugs close *K_{ATP}* channels independently of ATP, and hence are useful to treat certain forms of diabetes. By shielding an ER-retention motif in the C terminus of Kir6.2, SUR1 is essential for the plasma membrane expression of *K_{ATP}* (ref. 81). A loss of *K_{ATP}* currents should cause depolarization and insulin secretion. Severe hyperinsulinaemia was found with human loss-of-function mutations in either SUR1 (ref. 78) or Kir6.2 (ref. 79). By contrast, mutations that activate *K_{ATP}* should hyperpolarize β -cells, and thereby inhibit insulin secretion, leading to diabetes. Such mutations were found recently in individuals with neonatal diabetes⁸⁰. One mutation, R201H, changed a residue predicted to be involved in ATP-binding, and heterologous expression of this mutant showed a loss of sensitivity to ATP. Hence, higher levels of ATP (and ultimately glucose) are required for channel closure and insulin release, perfectly explaining the diabetic phenotype. Interestingly,

a common polymorphism in Kir6.2 that may slightly decrease its ATP-sensitivity was found more frequently in individuals with type II diabetes than in control populations⁸², suggesting that it is a risk factor for developing adult diabetes.

Intracellular ion channels in organellar acidification and Ca²⁺ metabolism

The acidification of compartments such as endosomes and synaptic vesicles depends on Cl⁻ currents that neutralize the charge transported by vesicular H⁺ ATPases. Without this neutralization, the voltage created by the ATPase would inhibit further proton pumping. The molecular identity of the corresponding Cl⁻ channels, however, was unknown until a human kidney stone disease⁸³ and mouse models^{84–86} suggested that *CLC*-type Cl⁻ channels are involved.

Dent's disease is an X-linked kidney stone disorder. It was surprising that the underlying gene encodes a Cl⁻ channel, *CLC-5* (ref. 83). The clue to the pathological mechanism came from the loss of low-molecular-weight proteins into the urine (proteinuria), another symptom of the disease. Small proteins pass the glomerular filter, and are endocytosed and degraded by proximal tubular cells. Proteinuria thus suggested a role for *CLC-5* in endocytosis (Fig. 3b). Immunocytochemistry identified *CLC-5* on apical endosomes of proximal tubular cells, where it colocalized with the H⁺ ATPase⁸⁷. *CLC-5*-knockout mice⁸⁴ have impaired apical endocytosis in proximal tubules. Fluid-phase and receptor-mediated endocytosis, and the endocytosis of plasma-membrane transport proteins, are also severely reduced in the mice, in a cell-autonomous manner⁸⁴. In proximal tubular cells, megalin, a member of the low-density lipoprotein receptor superfamily, is the main receptor for the apical endocytosis of proteins. The disruption of *CLC-5* in mice caused a cell-autonomous decrease in megalin, suggesting a role for this channel in recycling the receptor to the plasma membrane⁸⁴. As hypothesized earlier⁸⁷, the lack of *CLC-5* inhibited endosomal acidification^{84,87}, as shown with renal cortical endosomes *in vitro*⁸⁸.

The impairment of endocytosis explains proteinuria — but how does *CLC-5* disruption cause kidney stones? Parathyroid hormone (PTH) is freely filtered into the primary urine, and a decrease in megalin-mediated uptake of PTH increases luminal PTH in downstream nephron segments. The increased stimulation of apical PTH receptors in the proximal tubule negatively regulates the Na–phosphate cotransporter that is responsible for the bulk of phosphate reabsorption⁸⁴; excessive phosphate loss into the urine contributes to kidney stones. In addition, stimulation of the PTH receptor activates the transcription of α -hydroxylase, which converts vitamin D precursors into the active hormone⁸⁸. The former mechanism directly explains hyperphosphaturia, whereas changes in vitamin D metabolism may indirectly lead to hypercalciuria^{84,88}.

CLC-7 is the only *CLC* channel that is markedly expressed in lysosomes in addition to late endosomes⁸⁶. After osteoclasts attach to bone, *CLC-7* is inserted together with the H⁺ ATPase into a specialized plasma membrane domain called ruffled border (Fig. 3c). During this process, lysosomal enzymes are exocytosed into the adjacent resorption lacuna, which is sometimes referred to as the 'extracellular lysosome' and which is acidified by the ATPase. The acidic pH is needed for the activity of the lysosomal enzymes and for the chemical dissolution of the inorganic bone material. The disruption of *CLC-7* causes osteopetrosis by impairing the acidification of the lacuna, and hence bone degradation⁸⁶, in a mechanism that again may involve the compensation of proton pump currents. Once indicated by the mouse model, *CLC-7* mutations were then identified in human malignant infantile osteopetrosis⁸⁶ and in autosomal-dominant osteopetrosis type II (ref. 89).

Other CLC channels also participate in acidifying intracellular compartments. CLC-3, which is 80% identical to CLC-5, is expressed on endosomes and synaptic vesicles⁸⁵. Its disruption impaired synaptic vesicle acidification and caused severe neurodegeneration⁸⁵. The single yeast CLC Cl⁻ channel (scCLC or *Gef1p*)⁹⁰ resides in intracellular membranes that stain with Golgi markers^{91,92}. Similar to mammalian intracellular CLCs, scCLC could help in acidifying these compartments^{90–92}. It might also neutralize currents of a vesicular Cu²⁺ ATPase⁹². The associated changes in luminal Cl⁻ concentrations may have direct effects on enzymes⁹³.

Hence, a common theme for intracellular Cl⁻ channels is vesicular acidification, which has many roles in cell biology. For instance, receptor–ligand interactions are modulated by the increasingly acidic pH in the endocytotic pathway, lysosomal enzymes have acidic pH optima, and the electrochemical H⁺ gradient is used to drive neurotransmitter uptake into synaptic vesicles. Furthermore, in a poorly understood process, acidic endosomal pH is important for endocytotic trafficking. It is unknown how the information on endosomal pH is transduced to the cytoplasmic aspect of these vesicles. However, ARF6 and ARNO, which are regulators of endosomal trafficking, bind to endosomes depending on their luminal pH⁹⁴. The role of endosomal pH, and the diverse roles of distinct CLC channels in intracellular compartments, are challenging and fascinating problems for future research.

Besides vesicular Cl⁻ channels, two other intracellular channels, the RYR1 and RYR2 ryanodine receptor Ca²⁺ channels, are mutated in disease. Mutations in the *RYR1* gene were identified in pigs⁹⁵ and humans⁹⁶ with hyperthermia. This disorder is associated with life-threatening contractions of skeletal muscle due to uncontrolled Ca²⁺ release. Certain mutations in the cardiac isoform *RYR2* lead to a catecholamine-induced pathological increase in heart frequency⁹⁷ or to a form of cardiomyopathy⁹⁸.

Summary and outlook

Channelopathies have provided exciting and often surprising insights into the cellular function of ion channels. These diseases not only include neuromuscular and cardiac disorders — for which genes encoding ion channels were obvious candidates — but also pathologies as diverse as renal salt loss, diabetes, hypertension, kidney stones and osteopetrosis. The unravelling of underlying mechanisms highlighted the importance of ion channels in secretion and endocytosis, and revealed new regulatory pathways. Given the plethora of genes encoding ion channels, more surprises may be in store. □

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