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Authors
Goldstein, Steve AN
Bockenhauer, Detlef
O'Kelly, Ita
et al.

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Potassium Leak Channels and the Kcnk Family of Two-P-Domain Subunits

Steve A. N. Goldstein, Detlef Bockenhauer, Ita O'Kelly and Noam Zilberberg

With a bang, a new family of potassium channels has exploded into view. Although KCNK channels were discovered only five years ago, they already outnumber other channel types. KCNK channels are easy to identify because of their unique structure — they possess two poreforming domains in each subunit. The new channels function in a most remarkable fashion: they are highly regulated, potassium-selective leak channels. Although leak currents are fundamental to the function of nerves and muscles, the molecular basis for this type of conductance had been a mystery. Here we review the discovery of KCNK channels, what has been learned about them and what lies ahead. Even though two-P-domain channels are widespread and essential, they were hidden from sight in plain view — our most basic questions remain to be answered.

Potassium (K^+) leak currents have been described as essential to neuromuscular function for more than 50 years[1–4]. However, the existence of unique molecular transport entities responsible for leak currents (also called resting or background conductances) was questioned despite the fact that they were attributed prominent roles in the function of sympathetic ganglia[5,6], invertebrate axons[7], vertebrate myelinated axons[8–14] and cardiac myocytes[15–20].We now understand that leak is not just seepage, but flux through dedicated pathways.

Leak currents exert control over excitability by shaping the duration, frequency and amplitude of action potentials, in part through their influence over the resting membrane potential (BOX 1). Increased K^+ leak currents stabilize cells at hyperpolarized voltages below the firing threshold of nerves and muscles, whereas leak suppression permits depolarization and excitation. In addition, although leak channels pass K^+ readily upon changes in membrane voltage, they operate under tight control of agents as disparate as molecular oxygen, cyclic nucleotides, noradrenaline, serotonin and GABA (γ-aminobutyric acid)[21–24]. But despite their significant duties and tight regulation, native leak currents have defied coherent description for years. In a multitude of events occurring in just milliseconds, leaks seemed largely invariant; they were readily ignored, easily camouflaged and even actively removed by 'subtraction' during electrophysiological recordings. But cloning and study of TOK1 from *Saccharomyces cerevisiae*[25–35], KCNK0 from Drosophila melanogaster[36–38] and 11 mammalian two-P-domain K^+ channel genes have established unequivocally that leaks are indeed much more than unchanging dribble through pathways devoted to other functions (TABLE 1).

Box 1 | **What is leak?**

From the earliest days of electrophysiological recordings, a fixed conductance called the leak was used to explain the presence of a resting membrane potential from which action potentials emerged and to which they returned. In current parlance, 'leak' is still applied to fixed background currents present at rest and to currents that seem to rise instantly to a new steady level with voltage steps. Leaks can be non-selective (for example, when a membrane is damaged) or result from the movement of specific ions.

Even at rest, the plasma membrane of a living cell is bustling with activity: ions move in and out, and ionic and electrical transmembrane gradients are maintained. The ionic gradient is established by active transport systems (such as Na^+–K^+ pumps). These pumps expend cellular energy (ATP) to keep internal levels of Na+ low and K^+ high. Ion channels dissipate the ionic gradient by opening water-filled pathways across the membrane that allow for free diffusion of selected ions. An open K^+ channel allows K^+ ions to flow out of the cell down the K^+ concentration gradient. This leaves behind negative counter ions, producing an excess of negative charge inside the cell that limits K^+ flux: net outflow of K^+ continues until the chemical energy favouring efflux is balanced by the electrostatic energy favouring K^+ influx.At equilibrium,chemical and electrostatic forces are equal, efflux equals influx and there is no net movement of K^+ ions. The Nernst potential is the membrane voltage that yields equilibrium for a particular ion at a given ionic gradient. For a hypothetical membrane that allows only K+ to cross, the Nernst potential is the same as the equilibrium reversal potential for K^+ ions,EK, and is described by EQN 1:

$$E_{\mathrm{K}} = \frac{\mathrm{R}T}{z\mathrm{F}} \ln \frac{K_{\mathrm{ext}}}{K_{\mathrm{in}}} \qquad (1)$$

where R is the gas constant, *T* is temperature in degrees Kelvin, z is the charge of the ion, F is Faraday's constant and K_{ext} and Kin the concentrations of K^+ in the external and internal compartments, respectively. For physiological K^+ ion concentrations at physiological temperature (37° C),RT/zF is ~27 mV and EK is ~−97 mV. Mammalian cells have resting membrane potentials of −60 to −90 mV because K^+ ions are not the only ions that move at rest: for example, leak of Na+ and Cl− moves the membrane towards their Nernst potentials (about +67 and −93 mV, respectively). The resting membrane potential is often close to EK because more K^+ channels are open at rest than pathways for other ions.

Excitability depends on resting membrane potential because action potentials fire when the membrane voltage rises to a threshold level where voltage-gated channels open and subsequently become inactive. To fire again these voltage-gated channels must recover from inactivation by returning to a negative potential below threshold, that is, the resting membrane potential.

Table 1 | The two-P-domain potassium channels

Gene*	Subunit names‡	Species (chromosome)§	Expression mRNA‖ (**protein shown)	Phenotype¶	Regulatory influences#	Accession number‡‡	References
TOK1	TOK1	*S. cerevisiae* (X)		Outward	K1 Tx η, VA η	U28005	25–35
KCNKØ	KCNK0 dORK1	*D. melanogaster* (1–10A1–2)	CNS,SM, GU	Open	PKη	U55321	36–38
KCNK1	KCNK1 TWIK1 hOHO	*H. sapiens* (1q42–43) *M. musculus* (8) *R. norvegicus*	CVR, CNS, GU, GI CVR, CNS**, GU	None		U76996	48–50, 105,106
KCNK2	KCNK2 TREK1 TPKC1	*H. sapiens* (1q41) *M. musculus* *R. rattus*	CNS, GI CVR, CNS**, GU, SM	Open or voltage-dependent	PK ι, AA η, MS η, VA η Temperature η	AF00471	49,50,57, 58,69–72
KCNK3	KCNK3 OAT1 TASK1	*H. sapiens* (2p24.1–23.3) *M. musculus* (5B) *R. rattus*	CVR, CNS, GU, GI CVR** CVR, CNS**	Open	pHo ι, AA ι, LA ι, VA η	AF065163	61,75–86
KCNK4	KCNK4 TRAAK	*H. sapiens* (11q13) *M. musculus* (5)	CNS, GU CNS**	Open	AA η, MS η	AF247042	92,107–109
KCNK5	KCNK5 TASK2	*H. sapiens* (6p21.31–33) *M. musculus*	GU, GI UB	Open	pHo ι, VA η	AU028559	87,110
KCNK6	KCNK6 TOSS TWIK2	*H. sapiens* (19q13.1) *R. norvegicus*	CVR, CNS, GU, GI	Open	AA η, VA ι	AF134149	50,53, 111,112
KCNK7	KCNK7	*H. sapiens* (11q13)	CVR, CNS, GU, GI,	None		AF110522	52
KCNK8	Kcnk8	*M. musculus* (19,2B)	(**retina)			AF158234	51
KCNK9	KCNK9 TASK3	*H. sapiens* (8q24.3)	UB	Open	pHo ι, AA ι	AF212829	60,113,114
KCNK10	KCNK10 TREK2	*H. sapiens* (14q31) *R. norvegicus*	CNS, GU, GI	Open	AA η	AF279890	115,116
KCNK11		Reserved					
KCNK12	KCNK12 THIK2	*H. sapiens* (2p22–21) *R. rattus*	CVR, CNS, GU, GI	None		AF287302	73
KCNK13	KCNK13 THIK1	*H. sapiens* (14q24.1–3) *R. rattus*	UB	Open	AA η	AF287303	73
TWK-18	TWK-18	*C. elegans* (X)	Body wall muscle	Open	Temperature η	AAC32861	55
KCO1	KCO1	*A. thaliana*		Outward	Internal Ca^{2+} η	X97323	56

*Names for mammalian clones assigned by HUGO. KCNK and Kcnk represent human and mouse isolates, respectively. The 42 potential KCNK-like genes in *Caenorhabditis elegans*, including the first functional isolate (TWK-18), have not received KCNK designations.

‡KCNK and Kcnk represent protein isolates and are accompanied by other names used in the literature.

§Based on Northern blot or rt-PCR studies and categorized by system as CVR, cardiovascular; CNS, central nervous system; GU, genitourinary; GI, gastrointestinal; SM, skeletal muscle and integument; UB, ubiquitous.

‖*A. thaliana, Arabidopsis thaliana; D. melanogaster, Drosophila melanogaster; H. sapiens, Homo sapiens; R. norvegicius, Rattus norvegicius; R. rattus, Rattus rattus; S. cerevisiae, Saccharomyces cerevisiae*.

¶Outward, non-voltage gated outward rectifier; open, open rectifier.

#K1 Tx, K1 killer toxin; VA, volatile anaesthetic; PK, protein kinase; AA, arachidonic acid; pHo, lowered external pH; LA, local anaesthetic; MS, mechanical stretch. The criteria for listing as stimulatory (η) or suppressive (ι) were as follows: VA, ≥30% change to clinical concentrations of chloroform (≤0.8 mM), halothane (≤0.2 mM) and/or isoflurane (≤0.3 mM); PK, ≥ threefold change to activation or suppression of one or more protein kinase systems; AA, ≥50% change in response to ≤100 μM; pHo, ≥40% change within one unit of physiological pH (7.4); LA, ≥50% change in response to clinical concentration of lidocaine (≤300 μM) or bupivacaine (≤200 μM); MS, ≥ twofold increase in response to ≤–60 mm Hg; temperature, Q10 >4.

‡‡Human if available.

Potassium channels with two P domains

Before 1995,K^+ channel subunits were identifiable by the presence of a single pore-forming P domain, characterized by the amino-acid signature motif TXGYG[39,40]. For example, voltage-gated K^+ channel (K_v) subunits have one P domain and six transmembrane (TM) segments (1P/6TM). The fourth transmembrane domain (S4) acts as voltage sensor; it has positively charged residues at every third or fourth position and moves with changes in membrane potential to trigger channel opening[41,42] (FIG. 1a). Similarly, inwardly rectifying K^+ channel (Kir) subunits also have one P domain, but only two transmembrane segments (1P/2TM). The outward currents through these channels are relatively small owing to chronic obstruction by internal magnesium and POLYAMINES; when membrane voltage is negative with respect to the K^+ equilibrium potential (EK), K^+ ions that move into the cell relieve pore blockade and inward ion flux is significant[43–45] (FIG. 1b). In both families of one-P-domain channels, four subunits assemble to form a central K^+-selective pathway across the membrane[46,47].

In 1995, the first example of a two-P-domain channel subunit — TOK1 — was found in the sequence database for the budding yeast Saccharomyces cerevisiae; TOK1 has eight predicted transmembrane segments (2P/8TM) and constituted the first example of a non-voltage-gated outward rectifier[25–27] (FIG. 1c).The following year KCNK0 was cloned from the neuromuscular tissues of the adult fruitfly *Drosophila melanogaster* on the basis of its capacity to rescue K^+-transport-defective yeast cells[36]. KCNK0 subunits were also found to have two P domains but just four predicted transmembrane segments (2P/4TM) (FIG. 1d) and provided the first example of a canonical K^+- selective leak conductance channel. The first mammalian gene for a 2P/4TM subunit — KCNK1 — was also identified in the same year[39,48] and, although it was later proved to be a non-functional channel[49–51], attention was focused and these previously inconspicuous channel genes came rushing into view. So far, over 50 genes for 2P/4TM subunits have been recognized in sequence databases and 14 of them have been cloned, studied and formally designated (TABLE 1). Unfortunately, this frenzy of productivity has led to a nomenclature nightmare. So, there are identical subunits with multiple names; for example, TBAK1, TASK1, OAT1 and KCNK3 are the same subunit. In other cases, subunits are the product of non-homologous genes but share the same root name; for example, TASK1 (KCNK3) and TASK3 (KCNK9) are not significantly related to TASK2 (KCNK5). There are also identical genes with multiple names; for example, what some authors call Kcnk6 (REF. 52) is officially Kcnk8 (REFS 50,53).However, this is also unfortunate because Kcnk8 should have been Kcnk7, as it is the mouse variant of the human gene KCNK7 (REF. 52). There are also subunits named for functional attributes that later proved to be minor or inaccurately assessed. Moreover, the number of two-P-domain channels is expected to increase significantly on the basis of the presence of at least 42 potential genes for 2P/4TM subunits in the complete genomic sequence of the nematode *Caenorhabditis elegans*[25,54]. Indeed, one of them — TWK-18 — has now been shown to have K^+ channel activity55. In an attempt to avoid this confusion, we now use a simplified terminology that is gene-based and in accord with HUGO (Human Genome Organization) assignations; for example, KCNK0 gene and KCNK0 channel (TABLE 1).

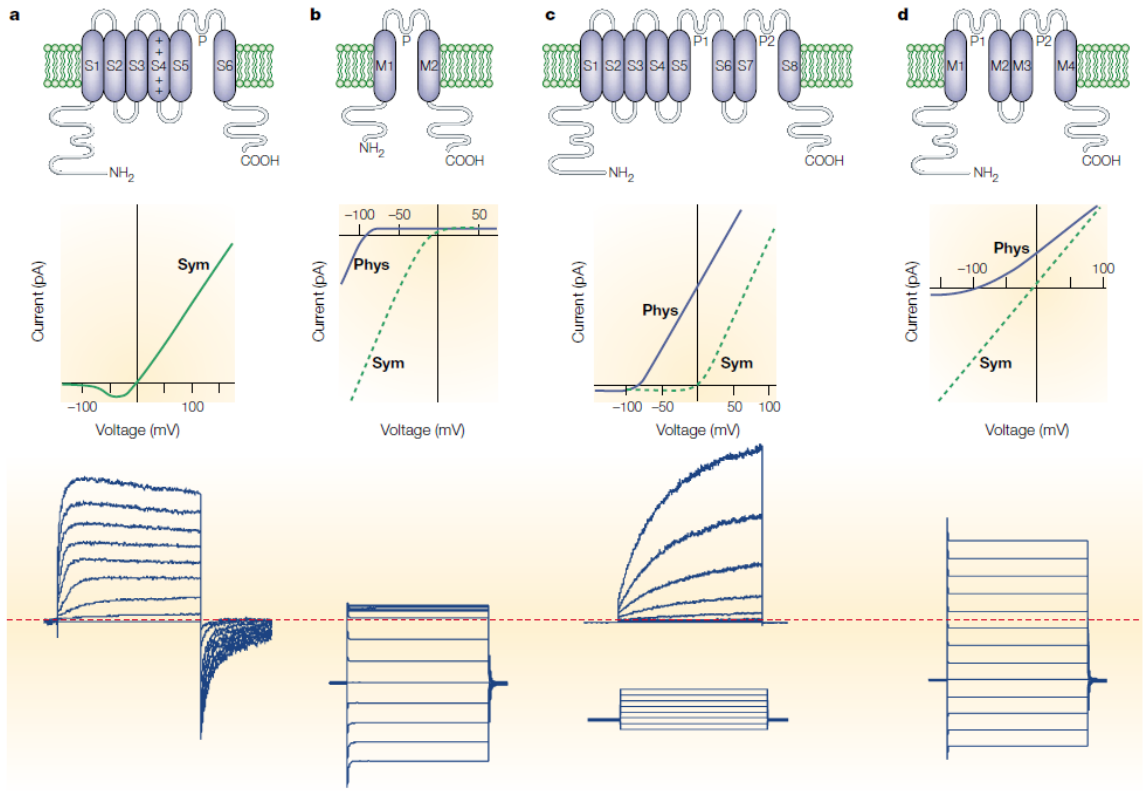

Figure 1 | **Potassium channels: membrane topology and current–voltage relationships.** Subunits are drawn with the external solution upwards. Graphs represent ideal examples. If the relationship is sensitive to changes in EK, two conditions have been drawn: symmetric (sym, dashed) and physiological (phys, solid) K^+ concentration. Currents are drawn based on channels studied in symmetric conditions with 500 ms pulses to voltages of −80 to 60 mV (c, bottom panel); the dashed line corresponds to 0 mV. **a** | Voltage-gated K^+ channel (Kv) subunits have a one-P/six transmembrane domain (1P/6TM) predicted topology and are noted for their positively charged fourth transmembrane segment (S4) that acts as a sensor for changes in membrane potential. Some Kv channels are activated by depolarization (as shown here), whereas others open in response to hyperpolarizing stimuli. The current trace is based on KCNQ1 and reveals a delay before current begins to flow, which reflects the time it takes for the channels to enter into the open state after the voltage change. **b** | Inwardly rectifying K^+ channel (Kir) subunits have one P domain and two transmembrane segments (1P/2TM). Kir channels pass small outward currents owing to chronic blockade by intracellular cations; large inward currents pass when voltage is negative to EK and the pore is unblocked. The current trace is based on Kir4.2 and reveals no delay before current begins to flow because unblock kinetics are faster than the resolution of the recording. **c** | TOK1, a non-voltage-gated, outwardly rectifying K^+ channel from *Saccharomyces cerevisiae* that has a 2P/8TM predicted topology. The channel passes outward current when membrane voltage is positive relative to EK. The trace reveals a fast and a slow phase to current development after a change in voltage. **d** | KCNK channels have a predicted 2P/4TM-

subunit topology. The channels are open rectifiers and allow the passage of large outward currents under conditions of high internal and low external K^+ (BOX 2). The current trace is based on KCNK0. It shows a nearly linear CURRENT–VOLTAGE RELATIONSHIP under symmetrical K^+, and reveals no delay before current starts to flow because the channels are open before the voltage step.

Box 2 | **Why do channels rectify?**

Potassium channels preferentially pass current in one direction (rectify) for three reasons:
• Unequal ion concentration.Current flows more easily from a side of high permeant ion concentration. This form of rectification, called Goldman–Hodgkin–Katz (or open) rectification, is seen with native currents[97,98] and cloned leak channels[36,38] (FIG. 1d), andexplains why KCNK channels pass outward current under physiological ionic conditions. So,KCNK0 has a linear single channel current–voltage relationship under symmetric conditions[38],whereas KCNK9 shows mild inward rectification[60]. Nonetheless, both channels pass significant currents only in the outward direction across the physiological voltage range under native ionic conditions of high internal and low external K^+. Although the underlying assumptions of electrodiffusion theory are inaccurate (ions do not move independently of each other or the pore), this theory predicts some of the observed nonlinear current–voltage relationships surprisingly well[36,38].
• Gating. Channels can rectify if they only open in a voltage range that favours outward or inward current. For example, some voltage-gated K^+ channels show voltage-dependent outward rectification because they open with depolarization to voltages where outward K^+ flux is favoured (FIG. 1a).Unlike TOK1, these channels do not shift their activation voltage with EK. Other depolarization-activated channels are inward rectifiers because they inactivate so rapidly once they open that they only pass large currents on recovery from inactivation at negative voltages where inward flux is favoured42. Still other voltage-gated channels show inward rectification because they are opened by steps to hyperpolarized voltages[99].
• Block. Some channels rectify because they are blocked in a voltage-dependent fashion. For example, inward rectifiers of the Kir class allow for the passage of inward current because internal magnesium and polyamines obstruct them when membrane voltage is positive to EK. The channels are unblocked at hyperpolarized potentials below EK, allowing significant inward K^+ flux[43–45] (FIG. 1b).

Functional types of two-P-domain channel

A dozen two-P-domain channel genes have been shown to function in experimental cells so far, and three have yet to show reproducible activity despite the presence of their transcript in native cells (TABLE 1). TOK1 and KCO1 (REF. 56) (a plant 2P/4TM subunit) encode non-voltage-gated outward rectifiers. The animal

isolates KCNK0, 2, 3, 4, 5, 6, 9, 10, 13 and TWK-18 encode K^+-selective leak channels; however, this does not seem to be their full functional repertoire, as at least one, KCNK2, can reversibly transform into a voltage-gated channel[57,58].

Outward rectifiers. TOK1 remains unique as the only 2P/8TM subunit. It functions as a non-voltage gated outward rectifier; that is, a channel that passes outward current in a K^+ concentration-dependent (and so EK-dependent) fashion (FIG. 1c), reminiscent of inward rectifier channels but in the opposite direction (FIG. 1b). TOK1 currents show an apparently instantaneous increase followed by a slow rising phase in response to depolarization[25,28,59]. In yeast cells (which are not noted for their excitability), TOK1 channels are a target of K1 killer toxin, a peptide encoded by an RNA virus that mediates strain-environmental dominance by killing its virus-free neighbours[33]. External K1 toxin directly activates TOK1 channels, leading to increased K^+ flux and death of virus-free cells. Conversely, internal K1 toxin blocks TOK1 channels, opposing the effect of external toxin and conferring immunity to virus-positive cells[35].Moreover, overexpression of TOK1 can rescue K^+-transport-defective yeast cells[32],whereas overactive channel mutants kill normal cells[59].Why K^+ homeostasis is crucial for yeast cell survival is uncertain, as resting potential and nutrient uptake in yeast cells are primarily dependent on a transmembrane gradient for protons rather than for K^+, as is the case in mammalian cells (BOX 1).

KCO1, a 2P/4TM channel from *Arabidopsis thaliana*, is also an outward rectifier but it responds both to changes in EK and in cytoplasmic Ca^{2+} levels[56]. The mechanism responsible for non-voltage-gated outward rectification (BOX 2) seems to involve both conformational changes of the protein and ion occupancy of the pore[25,26,28,31].

Open rectifiers — KCNK0. KCNK channels operate like K^+-selective holes in an electric field; that is, they show the attributes expected for leak channels.KCNK0 was the first clone to show the phenotype: MACROSCOPIC CURRENTS that seemed to be instantaneous, independent of voltage and selective for K^+ (REF. 36). Moreover, KCNK0 channels showed a behaviour called 'Goldman–Hodgkin–Katz rectification' or 'open rectification', as their conduction properties approximated predictions of constant-field current equations for free electrodiffusion through an open ion-selective pore[1,2]. In other words,KCNK0 currents change in a linear fashion as a function of voltage when the K^+ concentration is identical across the membrane. But when the concentration of K^+ is high intracellularly and low extracellularly (as occurs in

mammalian cells), a larger outward current is observed (FIG. 1d). Indeed, even KCNK channels that pass larger inward than outward single-channel currents in SYMMETRIC K^+ mediate primarily outward currents under physiological ionic conditions60; open rectification was an expected attribute of cloned K^+ leak channels (BOX 2).

Characterization of single KCNK0 channels has helped to define the basis for open rectification by showing, as expected, that leak currents accrue from channels that are open at rest[38]. So, voltage-gated K^+ channels show a pause before current flows

because they are closed before a stimulating voltage step; the delay reflects the time it takes the channel to move into the open conformation[41,42]. Conversely, single KCNK0 channels show bursts of activity that last for minutes (punctuated by long-lasting closures) at all voltages; as a result, the channels are often open before a voltage step and ready to pass current without delay[38] (FIG. 2a).Whereas all KCNK channels are expected to leak in this fashion, the various isolates have already begun to reveal their individual differences. For example, currents through KCNK0 rise instantaneously to a stable new level (FIGS 1a, 2a) whereas ion flow through Kcnk3 shows additional voltage- and time-dependent responses to changes in potential[61]. Studies of single KCNK0 channels have also shown that leak pores conduct ions like one-P-domain channels[36,38], showing rapid and selective ion flux through a pathway that holds multiple ions simultaneously [47,62–66]. So, KCNK0 channels show attributes that herald ion–channel and ion–ion interactions in a multi-ion pore[38]: nonlinear concentration-dependent changes in unitary conductance (FIG. 2b), ANOMALOUS MOLE-FRACTION BEHAVIOUR, pore occlusion by barium and a classical RELATIVE PERMEABILITY SERIES. This result was not surprising: the cloning of KCNK channels revealed that they used the same pore-forming motif as one-P-domain channels. However, significant differences are likely to exist between the pores of one-P and two-P-domain channels[67]. In the case of one-P-domain channels, four matching P loops are assembled to form the pore, whereas the two P domains in KCNK subunits are not identical[25,54].

Finally, studies of single KCNK0 channels have shown that leak channels are not always open.KCNK0 activity is linked strongly to protein kinase action; single channels open when kinases are active (open probability, Po ~1) and close when they are suppressed (Po <0.05)[37] (FIG. 2c). This does not herald all-or-none inflexibility; KCNK0 activity is finely tuned through integration of signals from multiple second-messenger pathways that involve protein kinases A, C and G. These pathways determine the frequency and duration of the long-lasting closed state37. The regulatory domain of KCNK0 seems to reside in its ~700 residue carboxyl terminus, as the region can be deleted to produce active but unregulated ~300 residue 2P/4TM channels (FIG. 2c) that contain the KCNK0 pore and its gate[37].

Like KCNK0 (and most classical one-P-domain K^+ channels[68]), the level of activity of other cloned KCNK channels is also strictly regulated. Notable influences include kinase-dependent pathways, arachidonic acid, membrane stretch, external pH and temperature (TABLE 1).

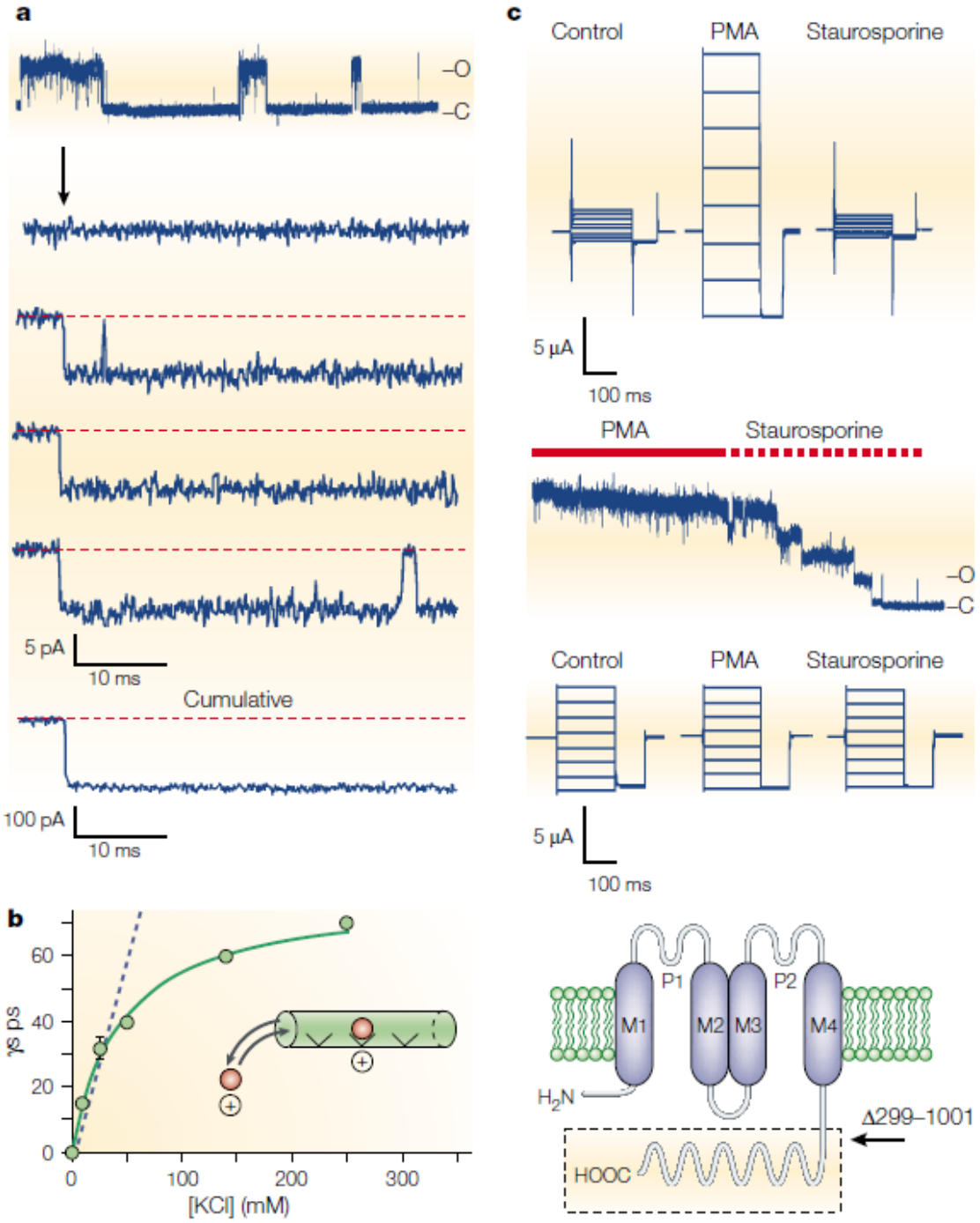

Figure 2 | **KCNK0 single channels open and close, show multi-ion attributes and are regulated**. KCNK0 channels were studied by expression in Xenopus oocytes. **a** | Top. Single KCNK0 channel recorded in an on-cell patch at 60 mV for 5 minutes with 140 mM KCl at the extracellular face of the channel; open state 3.3 pA. Bottom. Channel behaviour in response to a step from 0 to −120 mV (arrow). The first trace shows the response when the channel was closed (C) before the step; the next three traces show the response observed when the channel was open (O) before the pulse. (The dashed line is the 0 mV current level). Cumulative indicates an ensemble of 40 traces. **b** | KCNK0

single-channel conductance saturates with increasingly symmetrical K^+ (circles). Inset. Model of a multi-ion pore showing that ions entering a channel might find it occupied; as the new ion can not traverse the pore until the resident ion leaves, its movement is not independent (dashed line in top panel) and shows a saturating velocity. **c** | Top. Macroscopic KCNK0 currents measured by two-electrode voltage clamp in 20 mM K^+ with or without 50 nM PMA (phorbol-12-myristate-13-acetate) or 2 μM staurosporine. The cell was held at −80 mV and depolarized by 250 ms steps from −150 to 60 mV in 30 mV increments followed by 75 ms at −150 mV. Second panel. Four KCNK0 channels recorded in an on-cell patch at 60 mV with PMA in the bath and then staurosporine. Third panel. Macroscopic current traces for a cell expressing a channel without the carboxy-terminal 700 residues under conditions as in the top panel. Bottom. Model of a KCNK0 subunit showing the two functional domains, one pore-forming and the other regulatory. (Recordings adapted with permission from REFS 37,38.)

Phenotypic flexibility: KCNK2. Expressed robustly in the central nervous system (CNS), especially in the hippocampus, whole-cell KCNK2 currents are increased by arachidonic acid, mechanical stretch and volatile anaesthetics and diminished by lowered temperature via a protein kinase A (PKA)-dependent pathway[49,57,58,69–72]. Exploring the basis for PKA effects,we were surprised to find that regulation of KCNK2 could produce something other than more or less leak. Single KCNK2 channels show unexpected and dynamic functional versatility: reversible inter-conversion from a leak to voltage-dependent phenotype[57,58]. Thus,KCNK2 is an open rectifier when the single canonical PKA consensus site is mutated to alanine (or bears no phosphate), passing large currents both inwards and outwards in symmetrical conditions (FIG. 3a). Conversely, channels altered to aspartate at the site (or phosphorylated) pass more outward current even in symmetrical K^+ owing to voltage-dependent changes in their open probability (FIG. 3b). These channels show a half-maximal activation voltage >0 mV that does not change with EK (REFS 57, 58) and, so, are like classical voltage-gated channels (FIG. 1a; BOX 2) rather than the two-P-domain outward rectifiers TOK1 and KCO1 (FIG. 1c).This phenotypic alchemy is expected to enhance neuronal excitability as leak currents inhibit depolarization towards firing threshold, whereas channels activated at supra-threshold potentials do not interfere with rise to threshold but do facilitate recovery and repetitive re-firing.

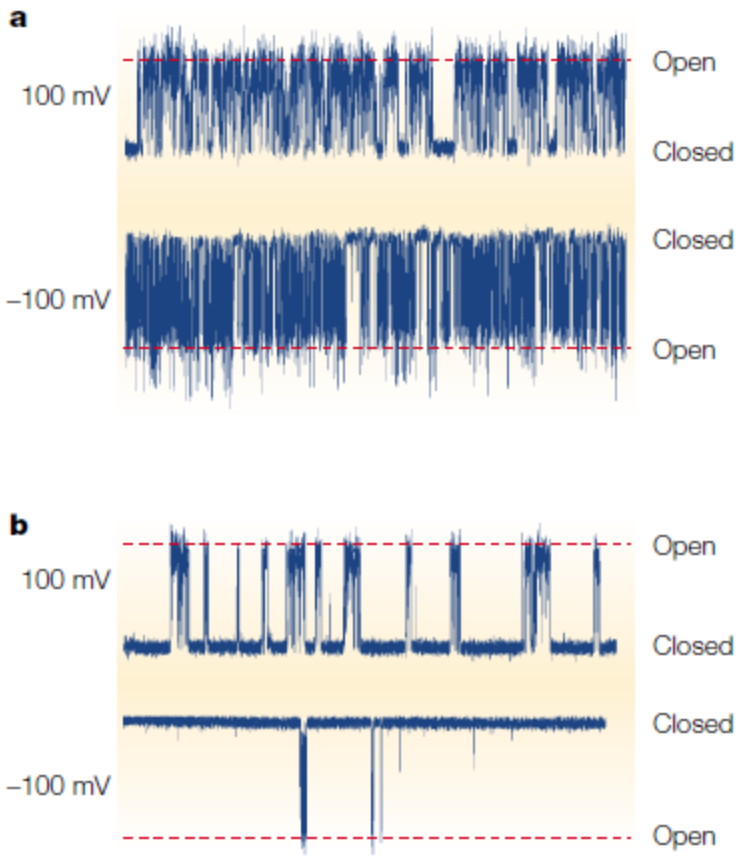

Figure 3 | **KCNK2: a leak pore that can also operate as a voltage-gated channel.** Inside-out patches were excised from oocytes expressing the channels and studied with symmetric 100 mM KCl; 2 s shown. Open state (dashed line) 7.8 pA at 100 mV and −8.5 pA at −100 mV; sampled 20 kHz, filtered 2 kHz (REFS 57, 58). **a** | Single KCNK2-S348A channels have the same open probability across a wide range of potentials as expected for a non-voltage-dependent leak pore. **b** | Single KCNK2-S348D channels show a greater open probability at 100 than −100 mV (~100-fold[57,58]).

The non-functional genes. The products of KCNK1, 7/8 and 12 remain silent despite reaching the plasma membrane[73]. We have proposed, therefore, that for these proteins to become functional channels, they require a hitherto unidentified accessory subunit, pore-forming subunit or regulatory influence[49,51]. So far, their expression with functional KCNK subunits has failed to provide evidence for heteromeric co-assembly[51,52,73], but the possibility that non-pore-forming subunits are required for the function of these proteins has received genetic support in C. elegans. A 2P/4TM channel subunit encoded by the SUP-9 gene is found in the same cells as two genetically interacting proteins that are predicted to reach the membrane and that lack P domains (REF. 74 and I. Pérez de la Cruz and H. R. Horvitz, personal communication).

Assigning KCNK channels to native leaks: KCNK3

Studies that seek to correlate KCNK genes and native currents are beginning to emerge in the literature, but such endeavours are difficult. Indeed, studies of one-P-domain channels have shown the common challenges to include, first, non-identical channels that behave similarly, and second, identical channels that behave differently as a function of their environment (for example, experimental versus native cells, two different cells in the same tissue, and the same cell type in health and disease). Such confounding variation results from altered gene expression, mRNA processing, subunit composition and channel modulation, and is also likely to slow identification of the physiological correlates of KCNK channels.

The process of establishing a correlation begins with a suspicion born of similarities between a native current and a cloned channel. It must then be confirmed that the channel protein is expressed in the cells of interest and, later, a direct biophysical comparison, ideally at the single channel level, should be done. In the case of K^+ leak currents, we begin from our expectations and encounter the first problem. Leak currents should be instantaneous, stable and serve to suppress excitation because they shift the resting membrane potential towards EK, a potential that is negative relative to the firing threshold of nerves and muscles (BOX 1).However, KCNK channels are not the only channel type to manifest these attributes: many classical one-P-domain channels also open in the hyperpolarized voltage range, influence resting membrane potential and contribute to K^+ leak (BOX 3).

Box 3 | **Do other K^+ channels leak?**

Yes. Any channel open at rest will contribute to background K^+ current. Classical one-P-domain channels that leak include:

• Kir subunits. The one-P/two transmembrane domain (1P/2TM) inward rectifiers act to stabilize cells near EK because their activity is dependent on inward flux of K^+ and relief from chronic channel block. An example is the acetylcholine-induced current in the heart, I_{KACh}, which is crucial for heart-rate control[100].

• Kv subunits. Some of these 1P/6TM subunits form channels that are active at hyperpolarized potentials when on their own[99].Others leak when associated with accessory subunits.For example,MinK-related peptide 2 (MiRP2) assembles with the Shaw family isolate Kv3.4 to form subthreshold K^+ channels in skeletal muscle that set the resting membrane potential and are associated in mutant form with inherited periodic paralysis[101].

• KCNQ subunits. These 1P/6TM subunits form mixed complexes of KCNQ2/3 and KCNQ3/4 that show a relatively hyperpolarized activation threshold (~−60 mV). They contribute to M CURRENTS in sympathetic neurons and the central nervous system, and are inhibited by muscarinic receptor activation and are associated with familial epilepsy[89–91].KCNQ1 subunits gain prominent expression in the ear and heart and are associated with inherited deafness and arrhythmia[91,102];MiRP2–KCNQ1 complexes have been shown to allow for the passage of K^+ ions at rest in experimental cells[103,104].

However, the task is not insurmountable, particularly when studies are facilitated by judicious use of the available pharmacological tools. Thus, the most extensively studied mammalian genes, rat Kcnk3 and mouse Kcnk3 (REFS 61,75–86) seem to mediate currents in several tissues. These include: cardiac background currents (I_{Kp}), neurotransmitter-inhibited currents in central neurons, neuronal currents altered by volatile anaesthetics and, perhaps, oxygen sensing by the CAROTID BODY84, and the action of ANGIOTENSIN II on adrenal cells[85].

Cardiac I_{Kp}. Kcnk3 is an open rectifier noted for its inhibition by external protons across the physiological pH range (FIG. 4a). In the mouse, Kcnk3 is expressed throughout the heart with prominence in the ventricles[61] (FIG. 4b). This channel shows low basal open probability (<0.05), brief transitions to the open state, blockade by acidification of the extracellular medium in a K^+-sensitive fashion, and decremental changes in activity in response to its most common regulators61. In cardiac myocytes, I_{Kp} influences the amplitude and duration of the action-potential plateau and, consequently, the duration of myocardial contraction[19]. On the basis of their common distribution and biophysical attributes, it seems likely that Kcnk3 channels contribute to native I_{Kp} currents in mouse heart[61,81].

Neurotransmitter-inhibited leak channels. Inhibition of resting K^+ leak currents is a widespread mechanism by which serotonin, noradrenaline, substance P, glutamate, thyrotropin-releasing hormone (TRH) and acetylcholine (acting through muscarinic receptors) enhance neuronal excitability in the nervous system11. This is crucial to the regulation of 'state-switching' in cortical and thalamic neurons, which seems to mediate transitions between sleep and wakefulness, and perhaps attentiveness[12]. Two groups have now associated KCNK3 with these transmitter-sensitive currents in rat hypoglossal motoneurons[83] and cerebellar granule cells[82]. Hypoglossal motoneurons express the KCNK3 transcript (FIG. 4c) and show KCNK3-like currents that are inhibited by lowered pH or by five different neurotransmitters known to operate through receptors that typically use the αq/11 G proteins. Once blocked by lowered pH, the native channels are no longer sensitive to the transmitters (FIG. 4d).Moreover, expression of the receptor for TRH with KCNK3 channels in experimental cells can reconstitute the phenomenon: TRH inhibits cloned KCNK3 currents unless they are previously suppressed by acidification[83] (FIG. 4d).

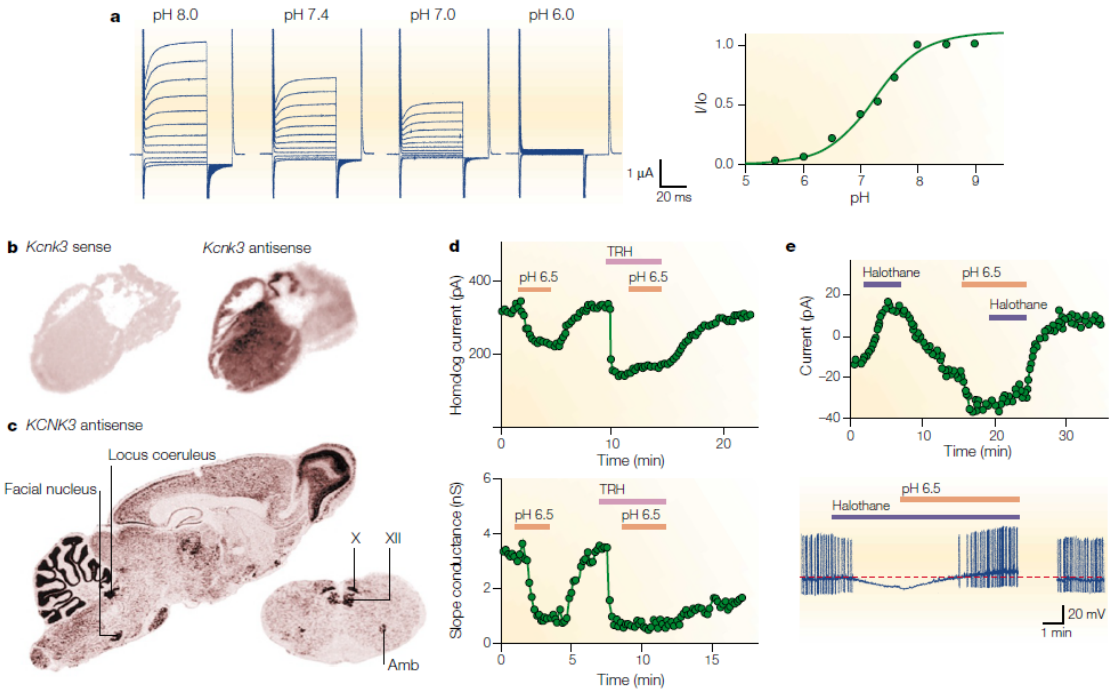

Figure 4 | **KCNK3 channels: molecular correlation with native cardiac and neuronal currents. a** | Cloned KCNK3 channels are inhibited by external protons at physiological pH. Two-electrode voltage clamp protocol: oocyte membrane potential was held at −80 mV and pulsed from −120 to +45 mV in 15 mV voltage steps for 50 ms, followed by a 20 ms step to −120 mV. A cell exposed to varying pH with 5 mM KCl bath solution is shown. The plot shows dependence of current at 0 mV on bath pH (mean ± SEM) for groups of five cells normalized to pH 8.0. The solid line represents a fit giving a pKa of 7.24 ±0.03 and a Hill coefficient of 1.02 ±0.06. **b** | In situ hybridization of mouse heart with an 35S-labelled antisense probe to mouse Kcnk3 shows a strong signal for message in ventricle and a weak signal in atria. **c** | In situ hybridization of rat brain with a 32P-labelled antisense probe to rat KCNK3 cDNA shows a strong signal for message in brainstem motoneurons — the nucleus ambiguus (Amb), and the facial, vagal (X), and hypoglossal nuclei (XII) — and in the locus coeruleus. **d** | Thyrotropin-releasing hormone (TRH) inhibits both KCNK3-like current in hypoglossal motoneurons and cloned KCNK3 channels in HEK 293 cells that also express the TRH receptor. Top. Hypoglossal motoneurons were exposed to low pH solution before and after changes in holding current induced at −60 mV by 1–2 μM TRH. Bottom. Slope conductance from ramp currents measured in HEK 293 cells expressing both KCNK3 and the TRH receptor when exposed to low pH solution before and after 0.1 μM TRH. TRH acted to inhibit the channel fully so that further effect was not observed by re-acidification. **e** | Halothane (0.3 mM) hyperpolarizes neurons of the rat locus coeruleus, suppressing firing by activating a KCNK3-like current. Top. Holding current of a cell at −60 mV is increased by halothane; the effect was inhibited by lowered pH. Bottom. Current clamp recording shows halothane-induced hyperpolarization and spike suppression that is reversed by acidification. (Panels **a** and **b** adapted with permission from REF. 61 © (2000) American Society for Biochemistry and Molecular Biology. Panels **c** and **d** adapted with permission

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Action of anaesthetics. Soon after they were identified, KCNK channels were considered prime candidates as native targets for anaesthetics[29,30].The idea was attractive because anaesthetics enhance leak currents in neural tissue (and therefore decrease excitability) and, at the same time, the presence of KCNK channels in the heart could explain the depressive side effects of anaesthetics. Several KCNK clones are sensitive to local and volatile anaesthetics (TABLE 1).Moreover, native KCNK3-like[86] and KCNK5-like channels[87] have been shown to respond at clinically relevant concentrations.KCNK3-expressing hypoglossal and locus coeruleus neurons (FIG. 4c) show halothane-induced, pH-sensitive membrane hyperpolarization that leads to decreased spiking activity[86] (FIG. 4e). Inhibition of hypoglossal and locus coeruleus neurons is thought to contribute to halothane-induced immobilization and to the analgesic and hypnotic actions of halothane, respectively.

These are compelling results, but caution is in order. One and two-P-domain K^+ channels that show comparable attributes are widely expressed in the heart and brain. Cardiac myocytes contain rapidly activating, noninactivating, voltage-dependent K^+ channels formed by 1P/6TM Kv subunits. These channels contribute to K^+ flux during the plateau of the cardiac action potential[88]. Similarly, voltage-dependent M channels formed by 1P/6TM KCNQ subunits might accompany neuronal, muscarine-inhibited KCNK3-like channels[82] as KCNQ channels are also suppressed by muscarine and external acidification, and contribute to resting K^+ flux owing to their activation at hyperpolarized potentials[89–91] (BOX 3). Furthermore, KCNK channels themselves show overlapping attributes and expression patterns. For example,KCNK2,KCNK3 and KCNK4 are neurotransmitter-inhibited resting currents that are coexpressed in several regions of the nervous system, although they do have distinguishable regulatory profiles[83,92,93] (TABLE 1). For instance, serotonin inhibits all three channels, but KCNK2 (like *Aplysia californica* sensory neuron S channels[21,94]) is suppressed via cyclic AMP and PKA, whereas KCNK3 and KCNK4 are responsive via another signalling pathway.

Many unanswered questions

Two-P-domain channels have been discovered at a remarkable rate over the past five years. This has produced a marvellous collection of KCNK genes waiting to be studied that seem well matched to the multitude of orphan channels without intrinsic voltage dependence that have been found in native cells[6,95]. Some long-standing issues have been addressed, but our most pressing questions remain to be answered. What roles do KCNK channels have in physiology and disease? Are they targets for common drugs? Where are their gates (and do they resemble the gates of one-P-domain channels as we suspect[28,33,34,37,59])? How does KCNK2 sense voltage? Will KCNK channels other than KCNK2 show phenotypic versatility? Do two-P-domain channels function as dimers (crosslinking through a non-conserved cysteine indicates that this might be the case[96])? Do both P domains contribute directly to pore formation? Will lack of identity between first and second P domains in KCNK channel subunits yield variations in gating or in ion selectivity67? Do KCNK subunits form heteromers? What kind of alchemy will bring the

non-functional KCNK variants to life?

Conclusion

Fifty years after K+-selective leak channels were first described, their genes have been found. They are numerous and widespread. The genes encode unique channel subunits with two pore-forming P domains. Whereas fungi and plants contain variants that operate as non-voltage dependent outward rectifiers, animals carry at least 50 genes for 2P/4TM subunits (the KCNK channels) that function as open rectifiers primarily passing outward current under physiological K^+ concentrations. Studies of single KCNK channels have confirmed that leak currents result from channels open at rest but have also shown that leak channels are not always open. Instead, like their native counterparts, the activity of KCNK channels is subject to strict regulation. As expected from classical studies, KCNK channels seem to control the excitability of nerves and muscles and, perhaps, to mediate the effects of volatile anaesthetics. Thus, neurotransmitter-mediated inhibition of resting K^+ flux has an excitatory influence, whereas increased activity with anaesthetic exposure stabilizes cells at rest. Regulation of one KCNK isolate has been observed to reversibly produce leak or voltage-dependent function, behaviours that stabilize cells or facilitate repetitive excitation, respectively. There is much yet to learn about KCNK channels now that they are visible. Like dark matter in the universe, the more we understand, the greater their influence seems to be.

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Contact:
Departments of Pediatrics and Cellular and Molecular Physiology,Boyer Center for Molecular Medicine, Yale University School of Medicine, New Haven, Connecticut 06536 USA. Correspondence to S.A.N.G. e-mail: steve.goldstein@yale.edu