Altered and dynamic ion selectivity of K+ channels in cell development and excitability

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Abstract

K^+ channels play a key role in regulating cellular excitability. It was thought that the strong K^+-selectivity of these channels was static, only altered by mutations in their selectivity filter, which can cause severe genetic disorders. Recent studies demonstrate that selectivity of K^+ channels can also exhibit dynamic changes. Under acidic conditions or in low extracellular K^+ concentrations, the two-pore domain K^+ channel (K_{2P}) TWIK1 becomes permeable to Na^+, shifting from an inhibitory role to an excitatory role. This phenomenon is responsible for the paradoxical depolarization of human cardiomyocytes in pathological hypokalemia, and therefore may contribute to cardiac arrhythmias. In other cell types, TWIK1 produces depolarizing leak currents under physiological conditions. Dynamic ion selectivity also occurs in other K_{2P} channels. Here we review evidence that dynamic selectivity of K_{2P} channels constitutes a new regulatory mechanism of cellular excitability, whose significance is only now becoming appreciated.

Keywords

channelopathies; resting potential; cationic channels; ionic pore

Ion selectivity of K+ channels

Rapid diffusion of ions through ion channels is essential for electrolyte homeostasis, cell development/differentiation and excitability. In physiological conditions, open K^+ channels allow K^+ to exit the cell, creating an excess of negative charges inside the cell and driving the membrane potential to hyperpolarized values. The opening of Na^+ channels has the opposite effect, allowing an inward rush of positive charges, which promote depolarization. Thus, at any moment, the excitability of the cell relies directly on the selectivity of the activated ion channels, with Na^+ influx favouring excitability and K^+ efflux opposing it. Genetic mutations leading to permanent alterations in the ion selectivity of K^+ channels are

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associated with severe disorders, further demonstrating how crucial K^+-selectivity is to cellular function [1–8].

Expressed in everything from viruses and bacteria to plants and animals, K^+ channels form the largest ion channel family. Despite their diversity, all these ion channels share the property of exhibiting highly selective transport of K^+ [9–11]. The signature sequence TxGY(F/L)G, which is conserved in the pore-forming loop (P-loop) of all K^+ channels, constitutes the core of the selectivity filter, which mainly determine the K^+ selectivity (Box 1) [10,11]. Mutations in the P-loop and its surrounding regions can affect ion selectivity [9,12]. This K^+ selectivity was originally thought to be static, independent of regulation by external stimuli. Recent studies have challenged this dogma by showing that K^+ channels exhibit dynamic ion selectivity in response to physiological and pathophysiological stimuli [13–15]. In the subsequent sections, we review the recent findings that have contributed to this new view that ion selectivity of K^+ channels can be both dynamic and regulated.

Gene mutations alter ion selectivity in K+ channels and result in disorders

A point mutation in the selectivity filter of Kir3.2 subunits permanently changes the ion selectivity and accounts for the phenotype of the *weaver* mouse. The neurological mutant mouse *weaver* has been studied for decades because of its particular neuronal development and neurologic disorders. The *weaver* mouse is profoundly ataxic because of the loss of granule cell neurons during cerebellar development [16]. This loss is due to a point mutation occurring in the gene encoding the G-protein-activated, inwardly rectifying K^+ channel protein, Kir3.2 (Figure 1). Kir3.2 (GIRK2, KCNJ6) forms functional heterotetramers with another member of the same channel family, Kir3.1 (GIRK1, KCNJ3) (reviewed in [17]). The *weaver* mutation (G156S) exchanges the first glycine residue for a serine in the selectivity sequence TxGYG of Kir3.2 [1,2]. Channels containing Kir3.2•G156S subunits lose K^+ selectivity, become nonselective cation channels, and constitutively conduct inward leak Na^+ currents. The Na^+ to K^+ relative permeability (P_{Na}/P_K) increases from ~0.03 for heteromeric Kir3.1/Kir3.2 wild type channels to ~0.94 for homomeric Kir3.2•G156S mutant channels and ~0.74 for heteromeric Kir3.1/Kir3.2•G156S mutant channels [2]. Kir3.1 and Kir3.2 are highly expressed in cerebellar granule cells during development, when *weaver* mouse granule cells are lost. The constitutive inward leak Na^+ conductance in *weaver* Kir3 mutant channels leads to excessive Na^+ influx, producing marked changes in intracellular Na^+ concentrations and increasing granule cell death [16].

Mutations in the selectivity filter and surrounding regions of Kir3.4 subunits also change the K^+ selectivity and are associated with human disorders. Kir3.4 (GIRK4, KCNJ5) is closely related to Kir3.2. In the heart, the heteromer Kir3.1/Kir3.4 underlies the cardiac muscarinic-gated channel (K_{ACh}) that contributes to the decrease in heart rate triggered by the activity of the parasympathetic nervous system [18]. Although the normal function of Kir3.4 in the adrenal gland is poorly understood, recent studies have shown that mutations in Kir3.4 cause aldosterone-producing adenomas (APAs) and inherited primary aldosteronism [3,4]. Two somatic mutations (G151R and L168R) in Kir3.4 were first identified by exome sequencing in APAs [3]. Further sequencing has found additional somatic mutations (E145Q; I157, deletion of the residue I157) and four inherited mutations (G151R, G151E, I157S, and

T158A) that are associated with APAs and familial primary aldosteronism [3–7]. E145 is located in the pore helix and G151 is the first glycine in the selectivity sequence TxGYG [9], the same glycine replaced by a serine in *weaver* Kir3.2 [1,2]. I157 and T158 are conserved among other Kir3 subunits and lie in the extracellular linker between the P-loop and the second transmembrane domain (TM2). L168 lies in the TM2, and its side chain abuts the highly conserved tyrosine side chain of the TxGYG motif (Figures 1B and 1C). All these mutations alter the ion selectivity of Kir3 channels. The P_{Na}/P_K values of heteromeric channels comprising Kir3.1 and Kir3.4•G151R, Kir3.4•T158A, and Kir3.4•L168R subunits are ~1, ~0.4, and ~0.77, respectively, as compared with ~0.04 for Kir3.1/Kir3.4 wild type channels (Figure 1D) [3]. Channels containing Kir3.1 and Kir3.4•E145Q (or Kir3.4•G151E or Kir3.4• I157 or Kir3.4•I157S) also conduct inward leak Na^+ currents [4,5,12,19], although their precise P_{Na}/P_K values have not been determined. These inward leak Na^+ currents cause cellular depolarization. Such depolarization is expected to activate voltage-gated Ca^{2+} channels, resulting in $Ca2^+$ entry into the cells, which in turn increases aldosterone production and cell proliferation of adrenal glomerulosa cells. Most interestingly, both G151E and G151R mutations occur at the same position, but G151E generates larger Na^+ conductance than G151R, sufficient to induce mammalian cell death [4]. Thus, G151E and G151R could cause different phenotypes in inherited aldosteronism, with severe hyperplasia in the one case and APAs in the other.

The last example of a genetic mutation known to cause an altered selectivity of the K^+ channel leading to a human syndrome relates to the HERG1 K^+ channel (KCNH2). The N629D mutation, which lies adjacent to the GFG signature sequence (GFG**N**), is associated with the cardiac long QT syndrome (LQTS). LQTS results from defects in K^+ and Na^+ channels that cause prolongation of cardiac repolarization and arrhythmias [20]. The tetrameric HERG1•N629D channel is non-selective among monovalent cations. Its P_{Na}/P_K value is ~0.65, as compared with 0.01 for the wild type channel. The N629D mutant channels replace the wild type outward repolarizing K^+ tail current with an inward depolarizing Na^+ current, which is expected to delay later stages of repolarization and contribute to arrhythmogenesis [8].

Hypokalemia induces changes in ion selectivity of TWIK1 that result in paradoxical depolarization in human cardiomyocytes

Hypokalemia is a common electrolyte disorder that occurs when blood K^+ concentrations are lower than the normal values of 3.5 to 4.8 mM. Whereas moderate hypokalemia (2.5–3 mM K^+) may lead to cardiac arrhythmias and other muscular disorders, more severe hypokalemia (less than 2.5 mM K^+) may cause cardiac arrest and sudden death [21,22]. The Na^+-K^+ pumps generate the physiological K^+ gradients across the cell membrane, which produce the hyperpolarized resting membrane potentials of cardiac cells. Background K^+ channels maintain the resting membrane potentials of cardiac cells at around −80 mV, close to the K^+ equilibrium potential [23]. Under hypokalemic conditions, in which the extracellular K^+ concentrations ($[K^+]_o$) are lower than normal physiological levels, many cardiac cells, including human cardiomyocytes and Purkinje fibers, exhibit three well-known aberrant behaviors [24–28]. First, their resting membrane potentials become

depolarized rather than hyperpolarized, which is inconsistent with the predictions made by the Nernst equation for K^+; second, their resting membrane potentials can become bi-stable; third, the recovery of the hyperpolarized resting membrane potential from the paradoxical depolarization after $[K^+]_o$ is restored to normal levels exhibits a kinetic hysteresis. Although some previous studies had implied that inward Na^+ currents might cause the paradoxical depolarization of cardiac cells [27,28], it was more generally believed that the paradoxical depolarization arose from the strong inward rectifier K^+ channels (Kir2), because these channels exhibit non-linear conductance near the K^+ equilibrium potential. They pass current more easily in the inward direction (into the cell) than in the outward direction (out of the cell). Because decreases in $[K^+]_o$ cause a progressive decline in the Kir2 conductance, Kir2 channels cannot fully compensate for the influence of the depolarizing inward currents [29,30]. However, this explanation could not account for several key observations related to the paradoxical depolarization of cardiac cells in lowered $[K^+]_o$, including bi-stability and the hysteresis of the recovery [30].

Data from our labs has now demonstrated that TWIK1 is involved in this phenomenon [13]. TWIK1 belongs to the family of two-pore domain K^+ channels (K_{2P}) that mediate background or leak K^+ conductance, and they participate in maintaining the resting membrane potential of many cell types. Each K_{2P} subunit contains two P-loops (P1 and P2) and 4 membrane-spanning helices (TM1 to TM4). K_{2P} channels are active as dimers (for a recent review on K_{2P} channels see [31]). TWIK1 mRNA is highly expressed in human atrium and Purkinje fibers [32,33], where paradoxical depolarization is often observed. In normal $[K^+]_o$, TWIK1 channels are highly selective for K^+, and their reversal potentials shift by ~53 mV in the depolarizing direction for every 10-fold increase in $[K^+]_o$, which is in agreement with the Nernst equation for K^+. In pathological hypokalemic conditions (when $[K^+]_o$ falls to lower than 3 mM), the reversal potentials are more depolarized than the values predicted by the Nernst equation (Figures 2B–2E). Thus, TWIK1 changes ion selectivity, becomes permeable to Na^+, and conducts inward leak Na^+ current under hypokalemic conditions. TWIK1 has a unique threonine residue (T118) in the selectivity sequence $TT^{118}GYG$ of the first P-loop, where other K_{2P} channels have a valine or isoleucine. Replacement of the threonine by isoleucine (T118I) eliminates the dynamic ion selectivity of TWIK1 channels (Figure 2D) [13].

The inward leak Na^+ currents through Na^+-permeable TWIK1 have a large impact on the resting membrane potentials. Mouse atrial-like HL-1 cardiomyocytes, which lack TWIK1, do not exhibit the paradoxical depolarization in sub-physiological $[K^+]_o$. However, when these same cardiomyocytes ectopically express TWIK1, they acquire the ability to undergo paradoxical depolarization in lowered $[K^+]_o$ (Figure 2F). Moreover, TWIK1 knockdown in human spherical cardiac myocytes significantly decreases their chances of showing the paradoxical depolarization in lowered $[K^+]_o$ [13]. These observations indicate that TWIK1 could indeed be responsible for triggering the paradoxical depolarization in human cardiomyocytes, influencing cardiac excitability through dynamic changes of ion selectivity under pathological, hypokalemic conditions.

The kinetics of the conformational changes between the K^+-selective and Na^+-permeable states of TWIK1 are very slow. The time to reach the maximal Na^+ permeability state takes

~10 min, with a time constant of ~373 s, and the time to recovery of the K^+ selectivity from the maximal Na^+ permeability is ~76 min (Figures 2G and 2H). Such a slow recovery of K^+ selectivity in TWIK1 suggests that these channels remain Na^+-permeable and able to conduct inward leak Na^+ currents for a prolonged period of time after $[K^+]_o$ is restored to normal levels. This behavior actually provides a biophysical basis for the hysteresis of restoring hyperpolarization from paradoxical depolarization and for bi-stable resting membrane potentials seen in sub-physiological $[K^+]_o$. Therefore, dynamic changes in ion selectivity of TWIK1 provide a reasonable explanation for three well-known behaviours of cardiac cells in lowered $[K^+]_o$.

TWIK1 ion selectivity is dynamically modulated by the external pH

The changing nature of the ion selectivity of TWIK1 also manifests under physiological conditions. In renal principal cells, for example, loss of TWIK1 expression leads to a decrease in cationic Na^+/K^+ conductance [34]. In pancreatic β cells, however, inactivation of TWIK1 leads to a hyperpolarization of the membrane potential rather than the expected depolarization, which is puzzling [15]. How could TWIK1 produce depolarizing currents at physiological K^+ concentrations?

In heterologous expression systems, the exogenous, newly synthesized TWIK1 is targeted to the plasma membrane, then becomes constitutively internalized and stored in recycling endosomes [35]. Mutation of a di isoleucine motif in the cytoplasmic Cterminal of TWIK1 prevents this endocytosis, resulting in more channels at the plasma membrane and measurable currents [36]. We took advantage of this mutant to reassess the regulation of TWIK1 by extracellular pH, a physiological parameter that modulates the activity of many other K_{2P} channels [37]. An initial report had suggested that TWIK1 was blocked by external acidification [38]. We found that although TWIK1 indeed displays a marked sensitivity to acidification, this sensitivity is biphasic (Figure 3A). TWIK1 is stimulated when pH falls from 7.5 to pH 6.5, but becomes inhibited when pH falls below 6.5. In TASK1/3 channels, which are related to TWIK1, which are related to TWIK1, the pH-sensor is a histidine residue following the TxGYG motif (TIGYG**H**) in the first P-loop [15]. This histidine is conserved in TWIK1, and its replacement by a neutral residue (H122N), which cannot be protonated upon acidification, completely abolishes TWIK1 channel sensitivity to acidic pH (Figure 3A). This result shows that histidine 122 is an essential component of the pH sensor.

In both wild-type TWIK1 and TWIK1•H122K, a marked shift of the reversal potential is observed upon acidification, suggesting a reduced selectivity for K^+. TWIK1•T118I. TWIK1•T118I remains K^+ selective in hypokalemic conditions and is insensitive to extracellular acidification, which suggests a link between TWIK1 inhibition by acidic pH and altered ion selectivity. To confirm the loss of K^+-selectivity at acidic pH, TWIK1 currents were recorded in different $[K^+]_o$ and the reversal potentials were plotted against $[K^+]_o$ (Figure 3B). At pH 7.4, the relationships for TWIK1 and TWIK1•T118I were linear, as expected for K^+-selective currents. At low $[K^+]_o$, however, only a slight deviation was observed for TWIK1, which corresponds to its altered selectivity in these conditions. At pH 6, the relationship for TWIK1 was strongly affected, even in high $[K^+]_o$, confirming the loss

of K^+-selectivity. Upon acidification, the P_{Na}/P_K value shifted from 0.03 to 0.19, confirming that TWIK1 conducts leak Na^+ currents upon acidification. The shift from the K^+-selective to the Na^+-permeable state was reversible but very slow (Figure 3D), exhibiting kinetics similar to those seen with decreases in $[K^+]_O$ (~373 s) [13] or with acidification (~266 s) [15], suggesting that they use similar mechanisms.

Residues in TWIK1 are important for its dynamic selectivity (Figure 3C and Box 1). TWIK1 has a unique motif, LFF, in the pore helix of the first P-loop, whereas other K_{2P} channels have FFF (TREK1, KCNK0) or FYF (TASK1) in the corresponding position. Whereas a TWIK1 mutant containing a FFF triplet behaves as the wild type channel, TWIK1 with FYF (TWIK1•L108F•F109Y) displays altered ion selectivity at both pH 6 and pH 7.4. TWIK1 also contains a leucine residue (L228) in the selectivity sequence TIG(F/L)G of the second P-loop, which is unique to the TWIK channel subfamily that also comprises TWIK2 and KCNK7 (Figure 4). However, mutating this leucine to a phenylanine, which reconstitutes the "classical" signature sequence found in the other K_{2P} channels, fails to abolish the dynamic selectivity of TWIK1. Stimuli modulating TREK channel activity converge on a common molecular gate comprising elements of the pore domains and the N-terminus of TM4 [39]. This gating element is conserved among K_{2P} channels and is employed regardless of whether the gating stimuli are inhibitory or stimulatory. In TWIK1, this element is a threonine (T250). In terms of selectivity TWIK1•T250L is more similar to TWIK1•T118I than it is to TWIK1 and retains K^+ selectivity at pH 6 (Figure 3C). The various types of K^+ channels, including K_{2P} channels, share a common molecular gate called the "C-type inactivation gate" or "upper gate". Originally described in voltage-gated K^+ channels (K_v), which spontaneously inactivate upon depolarization, this gating involves conformational rearrangements of the upper pore region leading to a closed state [40]. In many K^+ channels, the absence of external K^+ leads to a collapse of the pore in a closed state that is believed to be similar to the C-type inactivated state of K_v channels. For instance, before to enter in a stable non-conducting state, $K_V2.1$ switches from K^+-selective in the open state to Na^+-permeable during C-type inactivation [41]. Our results show that residues in P-loops and TM4 that are important for K^+ channel pore conformations and upper gating are also involved in the pH-dependent change of ion selectivity in TWIK1. Upon upper gate closure, TWIK1 would reach a Na^+-permeable state rather than a strictly closed state.

Taken together, these results indicate that TWIK1 can conduct Na^+ upon acidification. However, why would TWIK1 in renal principal cells and in pancreatic β cells behave as a Na^+/K^+ cationic channel at extracellular pH 7.4? The recycling rate of TWIK1 between acidic intracellular compartments and the cell surface may underlie its depolarizing or hyperpolarizing behaviour. During its transport along biosynthetic and recycling pathways, TWIK1 is exposed to intravesicular acidic pH. This condition is expected to promote a conformational arrangement of the selectivity filter of TWIK1 toward the non-selective state. When TWIK1 arrives at the cell surface it acts as a depolarizing Na^+/K^+ channel rather than as a hyperpolarizing K^+-selective channel. If TWIK1 spends several tens of minutes at the plasma membrane before endocytosis, it would eventually acquire K^+ selectivity and produce K^+ currents. This is certainly the case in both human cardiomyocytes

and *Xenopus* oocytes, explaining the original description of TWIK1 as a K^+-selective channel [42]. However, if TWIK1 is rapidly internalized, it would have insufficient time in the membrane to recover its K^+ selectivity. Its influence would then be depolarizing, which is what is observed in renal and pancreatic cells [15,34]. If true, this hypothesis would imply that the degree of influence of TWIK1 in the many tissues and cell types that express this unique channel depends on its recycling rate between endosomes and the plasma membrane.

Other K+ channels with permanently or dynamically-altered ion selectivity?

An alternative translation initiation site generates a truncated form of TREK1 that is permeable to Na^+ [43]. Recently, the TASK1/3 channels have been shown to change their ion selectivity upon acidification [14]. Sequence comparisons against TWIK1 suggest that many other K_{2P} channels should exhibit dynamically or permanently altered ion selectivity (Figure 4). A polymorphism occurring at high frequency (60%) in the human gene encoding TASK5 has the unusual sequence EYG in P1 instead of the classical GYG motif (Figure 4) [44,45]. Also the human KCNK7 channel contains a GLE sequence in P2, whereas the mouse KCNK7 contains GLG at the equivalent position. These variants, EYG and GLE, are incompatible with a K^+-selective pore (Box 1). Thus, depending on the expressed polymorphism, TASK5 channels might behave as depolarizing channels in some individuals and as hyperpolarizing channels in others, which is rather puzzling. Equally puzzling is the possibility that the ion selectivity KCNK7 be radically different in the two mammalian lineages. This rather suggests that all these channels either are inactive or active but with a permanently altered ion selectivity. Unfortunately this prediction has not yet been tested, since when expressed in heterologous systems, TASK5 and KCNK7 are mainly detected in intracellular compartments rather than in the plasma membrane, where their electrophysiological properties could be tested. When the GLE mutation is introduced in TWIK1, which is highly homologous to KCNK7, TWIK1 becomes permeable to Na^+, suggesting that KCNK7 is a non-selective Na^+/K^+ channel (unpublished result).

Singular sequences in pore domains are not restricted to mammalian K_{2P} channels. *Caenorhabditis elegans* has more than 40 genes encoding K_{2P} channels, making this class of K^+ channels the most abundant in the nematode [46]. Indeed, a dozen worm K_{2P} channels have uncommon motifs. Figure 4 shows an alignment of pore helices and selectivity filters of these channels. Most of them are mainly expressed in excitable cells, including neurons (TWK-3, TWK-4, TW-17), interneurons (TWK-16, TWK-32) and excretory cells (TWK-36) [47]. With a threonine equivalent to TWIK1 T118 in P1 and a GLG triplet in P2, the nematode TWK-25, TWK-34 and TWK-36 channels are very similar to mammalian TWIK1, making them very good candidates for channels with dynamic ion selectivity. However, it should be noted that they lack the histidine in the first P-loop, which is the pH-sensor in TWIK1. TWK-17, which is related to the channels mentioned above, does have a histidine at this position, but also displays a very unusual first pore domain with a GYD motif instead of the classical GYG, suggesting that ion selectivity is permanently altered. TWK-3, TWK-4, TWK-10, TWK-32 on the one hand, and TWK-6, TWK-16, TWK-29 on the other hand, are also quite unusual among K_{2P} channels. Instead of having the strictly conserved threonine located two residues upstream of the signature sequence in one of their P-loops (TXGXG), both groups of channels have another amino acid, either a serine or a

proline, for the first group and an alanine or a leucine for the second group. Given the role of the threonine side chain in K^+ coordination (Box 1), its substitution by other residues is expected to alter ion conduction. In addition, the second group of channels displays uncommon signature sequences in P2, suggesting also a non-selective state. TWK-16 and TWK-29 also have substitutions in their signature sequences (GYN in P1 and DFG in P2 for TWIK-16 and EYG in P2 for TWK-29) that indicate a possibly altered ion selectivity. Possessing a combination of two GYG motifs within the same subunit, TWK-44 is also very interesting. This combination is never seen in mammalian K_{2P} channels, and when introduced into mammalian TWIK1, it produces a non-functional channel [15]. Cloning and heterologous expression of *Caenorhabditis elegans* channels should allow these predictions to be tested through electrophysiological characterization and determination of their ion selectivity.

Concluding remarks

K^+-selectivity is a hallmark that characterizes a wide class of inhibitory ion channels present from virus and bacteria to plants and animals. Recent data have revealed that an alteration of this property is not only related to deleterious gene mutations but also seen in both pathological and physiological conditions. Future work will address the physiological relevance of the dynamic ion selectivity of TWIK1, and particularly its role in inhibitory and excitatory processes in the many tissues and cell types that express this channel. Cloning and characterization of the ion selectivity of channels related to TWIK1 in mammals and *Caenorhabditis elegans* should help to verify that dynamic ion selectivity is not a unique event in evolution but rather a property shared by multiple K^+ channels that has been overlooked. Ion channels are major targets of the current pharmaceutical drugs. These drugs that modulate channel activity and cell excitability have proven to be useful as anxiolytics, neuroleptics, anticonvulsants, local and general anesthetics, pain killer as well as cardiac antiarrhythmics. If dynamic selectivity proves to be more common than expected, designing drugs that modulate ionic selectivity may constitute a new avenue in therapeutic intervention.

ACKNOWLEDGMENTS

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Box 1

Ion selectivity in K$^+$ channels

Four TVGYG signature sequences form the selectivity filter of the KcsA channel (Figure IA). In the conduction pathway, dehydrated K$^+$ ions are stabilized by negatively charged groups and translocate in a single file [11]. Side-chains of the first threonines and the backbone's carbonyl groups point toward the central axis of the pore, creating cage-like K$^+$ binding sites, which are noted S1 to S4. This unique environment stands in for the hydration shells of solvated K$^+$ ions (Figure IB) [10] and establishes favorable structural constraints for both selectivity and fast translocation. In the selectivity filter, other ion binding sites located in the planes of the carbonyls have been suggested, in which smaller ions, such as Na$^+$ or Li$^+$, would be more efficiently coordinated [48–50]. K$^+$ within the conduction pathway is required to preserve a high rate and selective conductive conformation more favorable for K$^+$ than for Na$^+$ [51,52]. The large energy barrier formed by the concomitant presence of two K$^+$ ions at their binding sites would prevent Na$^+$ to access their own binding positions in the carbonyl planes (Figure IC). The structures of KcsA channels at low K$^+$ concentrations exhibit changes in the conduction pathway and nearby vicinity, showing a collapse of the pore [10]. This rearrangement results in a non-conductive conformation of the filter. However, some K$^+$ channels exhibit Na$^+$ permeability in the absence of K$^+$ [53–55], suggesting that perturbations in the selectivity filter geometry can also generate pores permeable to smaller ions. In KcsA, residues in the selectivity filter and the adjacent pore helix domain form, with water molecules, a complex network of hydrogen bonds, salt-bridges and electrostatics interactions [10,56,57] (Figure ID) that determine the stability of the selectivity filter [57,58]. Similar interactions exist in or are predicted for other K$^+$ channels [40,59,60]. Depending on their nature and strength, the channel would be susceptible to collapse in a non-conductive state or in a state permeable to smaller ions.

Highlights

- K^+ channels produce K^+ influx that decreases cell excitability

- Gene mutations altering K^+-selectivity of K^+ channels produce disorders in mouse and human

- TWIK1 K^+ channels become permeable to Na^+ upon hypokalemia

- TWIK1 K^+ channels become permeable to Na^+ upon acidification

- This dynamic selectivity relies on specific residues in the pore domain

- Sequence conservation suggests that dynamic selectivity has been conserved during evolution
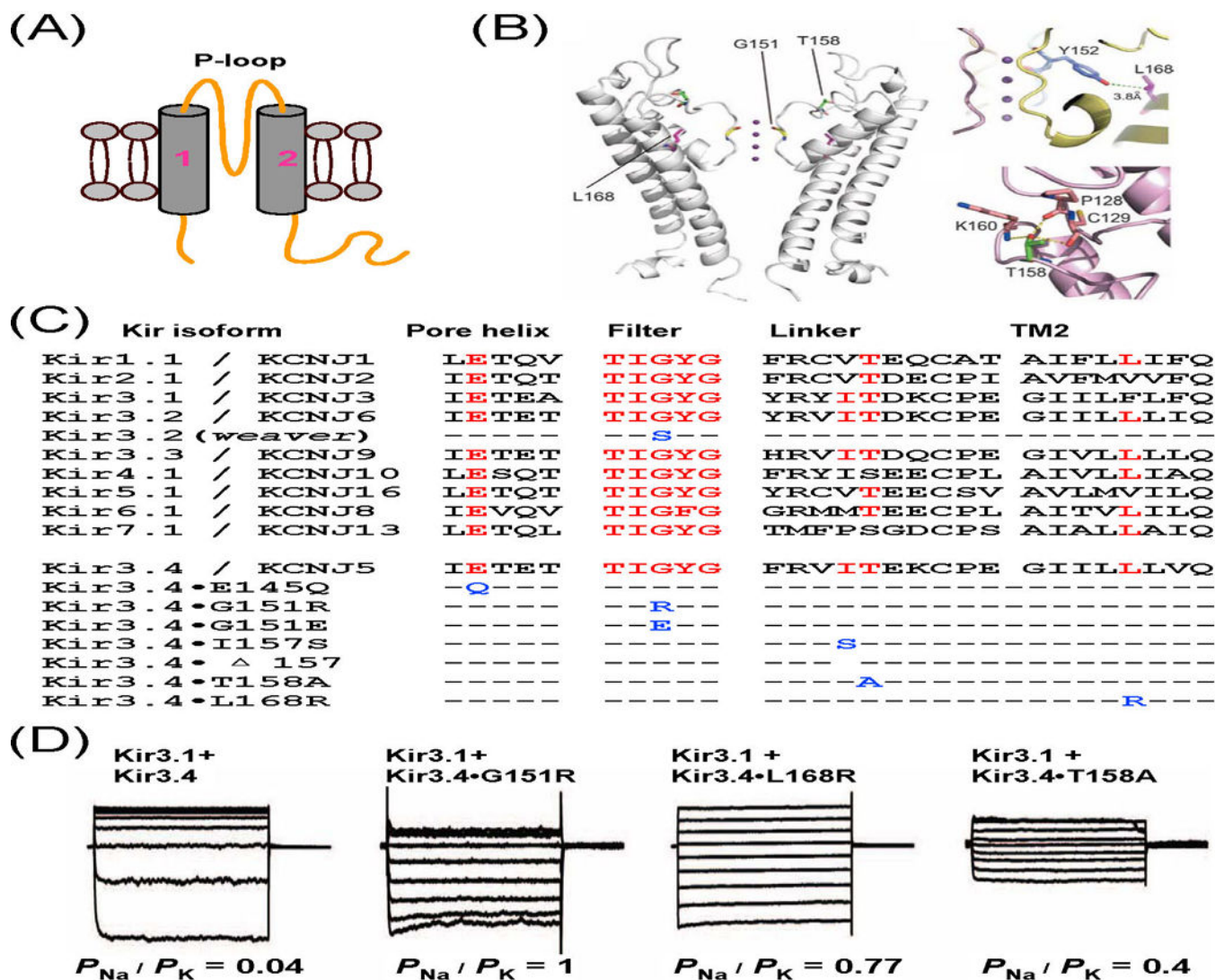
(A) P-loop

(B) G151 T158 ... Y152 L168 3.8Å ... P128 C129 K160 T158 L168

(C)

Kir isoform		Pore helix	Filter	Linker	TM2
Kir1.1	/ KCNJ1	LETQV	TIGYG	FRCVTEQCAT	AIFLLIFQ
Kir2.1	/ KCNJ2	IETQT	TIGYG	FRCVTDECPI	AVFMVVFQ
Kir3.1	/ KCNJ3	IETEA	TIGYG	YRYITDKCPE	GIILFLFQ
Kir3.2	/ KCNJ6	IETET	TIGYG	YRVITDKCPE	GIILLLIQ
Kir3.2 (weaver)		-----	--S--	----------	--------
Kir3.3	/ KCNJ9	IETET	TIGYG	HRVITDQCPE	GIVLLLLQ
Kir4.1	/ KCNJ10	LESQT	TIGYG	FRYISEECPL	AIVLLIAQ
Kir5.1	/ KCNJ16	LETQT	TIGYG	YRCVTEECSV	AVLMVILQ
Kir6.1	/ KCNJ8	IEVQV	TIGFG	GRMMTEECPL	AITVLILQ
Kir7.1	/ KCNJ13	LETQL	TIGYG	TMFPSGDCPS	AIALLAIQ
Kir3.4	/ KCNJ5	IETET	TIGYG	FRVITEKCPE	GIILLLVQ
Kir3.4•E145Q		-Q---	-----	----------	--------
Kir3.4•G151R		-----	--R--	----------	--------
Kir3.4•G151E		-----	--E--	----------	--------
Kir3.4•I157S		-----	-----	----S-----	--------
Kir3.4• △ 157		-----	-----	---△------	--------
Kir3.4•T158A		-----	-----	-----A----	--------
Kir3.4•L168R		-----	-----	----------	----R---

(D)

Kir3.1+ Kir3.4 — $P_{Na}/P_K = 0.04$

Kir3.1+ Kir3.4•G151R — $P_{Na}/P_K = 1$

Kir3.1 + Kir3.4•L168R — $P_{Na}/P_K = 0.77$

Kir3.1 + Kir3.4•T158A — $P_{Na}/P_K = 0.4$

Figure 1. Somatic and inherited mutations alter ion selectivity of G protein-gated K^+ channels
(**A**) Topology of Kir subunits with two transmembrane domains (TM1 and TM2) and one P-loop. (**B**) Residues G151, T158, and L168 of Kir3.4 are located respectively in the selectivity filter, the linker and TM2. (**C**) Sequence alignment of P-loops and upper parts of TM2 of human Kir channels and mouse Kir3.2 channel. Conserved residues are labelled in red, and mutations in Kir3.2 and Kir3.4 in blue. (**D**) Whole-cell currents recorded in HEK293T cells and corresponding P_{Na}/P_K values. (**B** and **D** adapted from [3]).
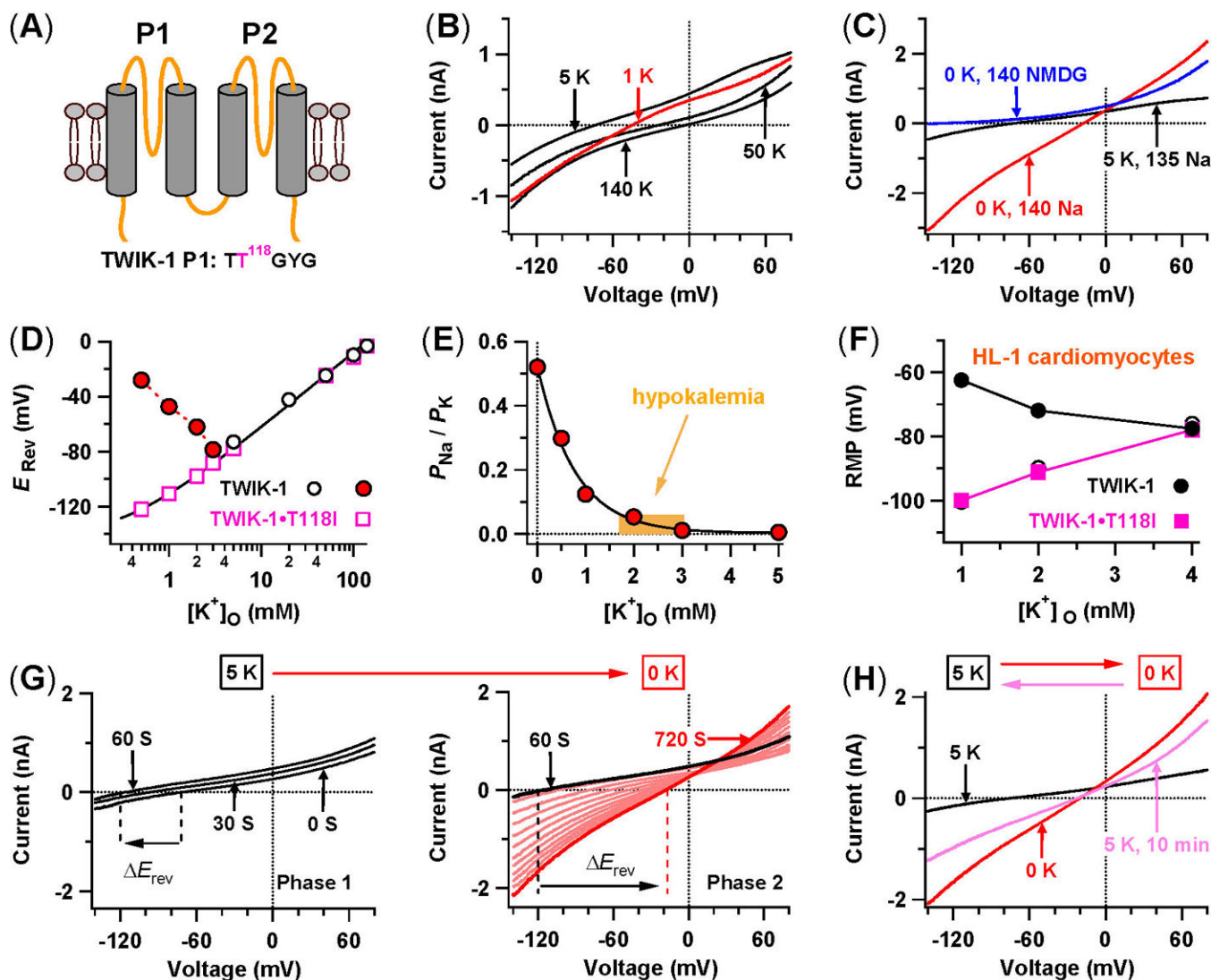
Figure 2. In hypokalemic conditions, TWIK1 K⁺ channels conduct inward leak Na⁺ currents and induce cardiac paradoxical depolarization

(**A**) TWIK1 topology with four transmembrane domains (TM1 to TM4) and two P-loops (P1 and P2). The sequence of its K⁺ selectivity sequence TTGYG in P1 is shown. (**B**) Whole-cell TWIK1 currents in transfected CHO cells in different external K⁺ concentrations. (**C**) Direct recordings of inward leak Na⁺ currents. Whole-cell TWIK1 currents are shown while Na⁺-based bath solutions with 5 mM K⁺ are shifted to 0 mM K⁺, and finally to an NMDG⁺-based bath solution with 0 mM K⁺. (**D**) Reversal potentials (E_{rev}) measured in Na⁺-based bath solutions with various $[K^+]_O$. The continuous line is a fit for open squares with the Goldman-Hodgkin-Katz voltage equation. (**E**) P_{Na}/P_K values in normal and low $[K^+]_O$. (**F**) Resting membrane potentials (RMP) of mouse HL-1 cardiomyocytes with and without over-expression of TWIK1 and TWIK-1•T118I in 1, 2, and 4 mM $[K^+]_O$. (**G**) Two-phase changes of whole-cell TWIK-1 currents are shown while Na⁺-based bath solutions are changed from 5 mM to 0 mM K⁺. (**H**) Whole-cell TWIK1 currents are shown when a Na⁺-based bath

solution is reversibly changed from 5 mM to 0 mM K$^+$, and then back to 5 mM K$^+$ for 10 minutes. (adapted from [13]).
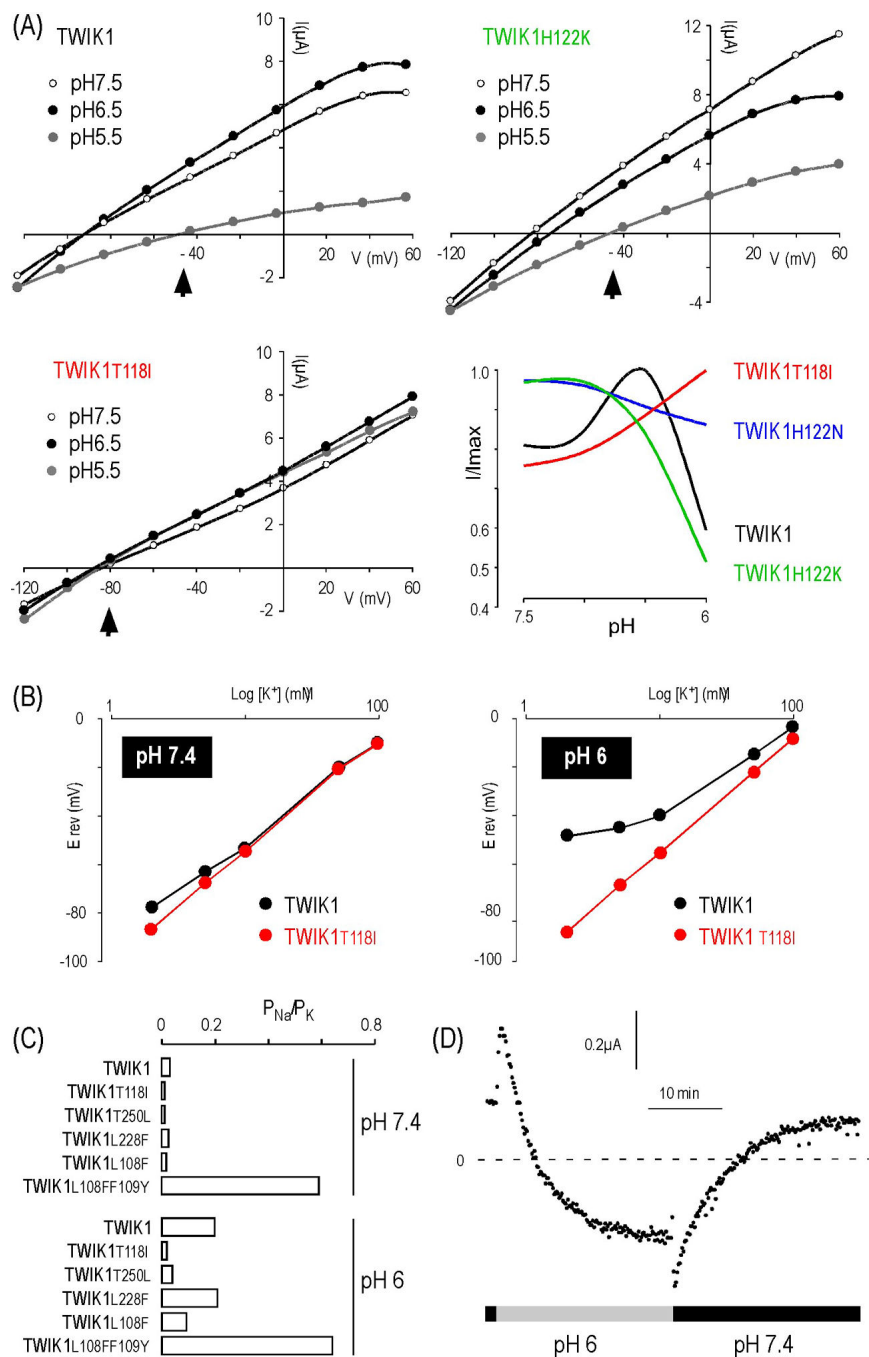
Figure 3. TWIK1 ion selectivity is regulated by extracellular pH

(**A**). Sensitivity of TWIK1 and TWIK1 mutants to acidification. The shift of the reversal potential (E_{rev}) at pH 5.5 is indicated by an arrowhead. (**B**). E_{rev} as a function of the extracellular K^+ concentration at pH 7.4 and 6.0. (**C**). P_{Na}/P_K values of different TWIK1 mutants at pH 7.4 and 6.0. (**D**). Kinetics of the ion selectivity change. TWIK1 currents were recorded at −80mV (adapted from [15]).
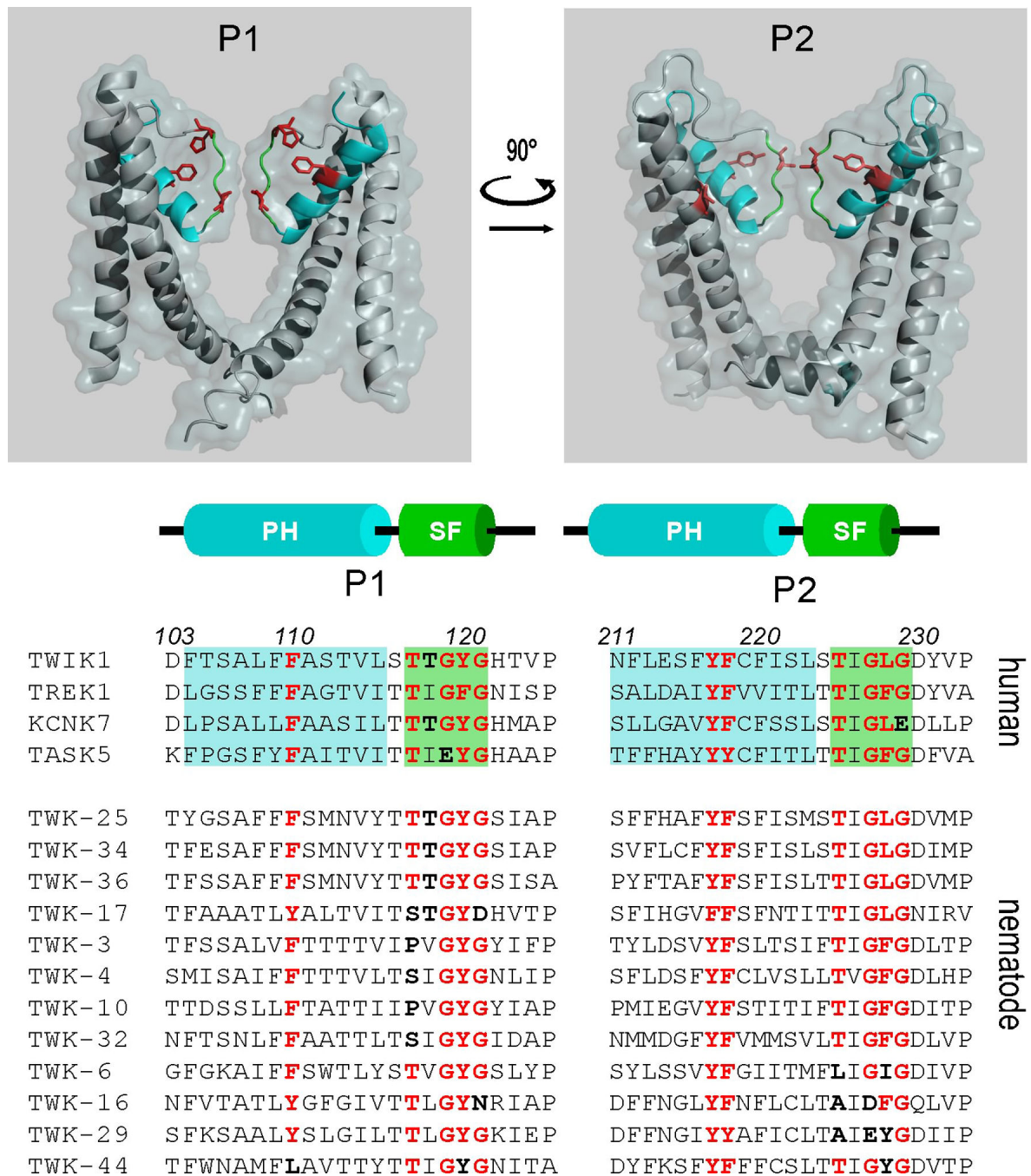
Figure 4. Human and nematode K₂ₚ channels show unusual sequences in their Ploops
Upper panel: Regions constituting the pore helix and the selectivity filter are shown in the crystal structure of TWIK1. Extracellular and intracellular domains of TWIK1 are not included. Pore helices (PH) are in blue and selectivity filters (SF) are in green. The side chains of residues affecting ion selectivity (L108, F109, T118, H122, L228, T250) are in red. Lower panel: sequence alignment of the pore domains of human and *Caenorhabditis elegans* K₂ₚ channels. Unusual residues are in dark bold.

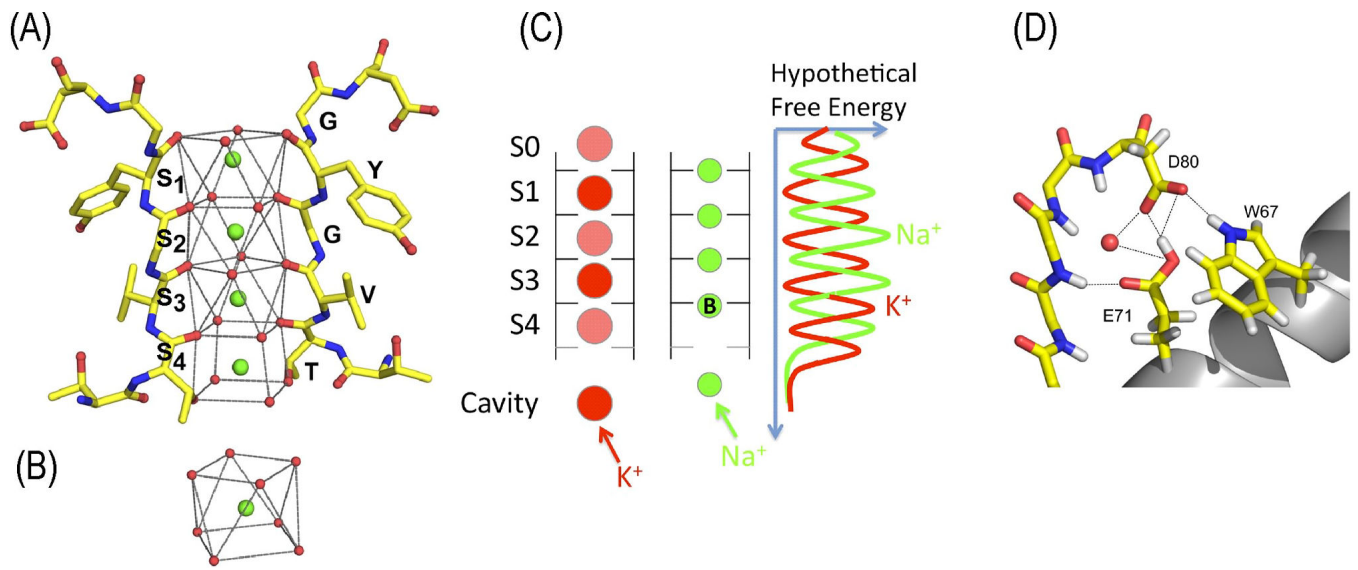**Figure I. Ion conduction pathway in K$^+$ channels**