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Review

Structural correlates of selectivity and inactivation in potassium channels ☆

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ABSTRACT

Potassium channels are involved in a tremendously diverse range of physiological applications requiring distinctly different functional properties. Not surprisingly, the amino acid sequences for these proteins are diverse as well, except for the region that has been ordained the "selectivity filter". The goal of this review is to examine our current understanding of the role of the selectivity filter and regions adjacent to it in specifying selectivity as well as its role in gating/inactivation and possible mechanisms by which these processes are coupled. Our working hypothesis is that an amino acid network behind the filter modulates selectivity in channels with the same signature sequence while at the same time affecting channel inactivation properties. This article is part of a Special Issue entitled: Membrane protein structure and function.

Contents

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1. Introduction

In this manuscript we discuss the role of the selectivity filter region in determining both selectivity and inactivation (a non-conductive state of the channel, distinct from a closed state) from the perspective of the prokaryotic potassium channel KcsA. We focus on three different families of potassium channels: the voltage-gated potassium channels, the inward rectifying potassium channels, and the calcium-activated potassium channels.

Voltage-gated potassium (Kv) channels are members of the S4 superfamily of ion channels [1,2], each subunit is characterized by 6 transmembrane helices (S1–S6), a voltage sensor within helices S1 through S4 (with positively charged amino acids every 4th position in S4), and a pore region between S5 and S6 (Fig. 1A). Kv channels are conserved from bacteria to humans, are ubiquitously expressed, and have a multitude of physiological roles.

Inward rectifier potassium (Kir) channel subunits have only two transmembrane helices flanking a pore region, corresponding and homologous to the S5 and S6 segments in Kv channels (Fig. 1B). Kir channels also possess a cytosolic domain composed of both the N- and C-termini of the channel that helps regulate channel gating [3].

Calcium-activated potassium (K_{Ca}) channels are also members of the S4 superfamily of ion channels. The large conductance Ca^{2+}-activated K^+ channels (BK, MaxiK, $K_{Ca}1$) are both voltage and Ca^{2+} activated (Fig. 1C). Ca^{2+} activation occurs via Ca^{2+} binding to multiple sites on the cytosolic region of this channel [4,5]. Conversely, the small conductance Ca-activated K^+ (SK, $K_{Ca}2$ and 3) channels are not voltage gated (despite possessing a voltage sensor-like domain) and Ca^{2+} activates them via binding to calmodulin, which is constitutively bound to the cytosolic portion of the channel [6–9].

All of these channels share a similar pore architecture where the acute selectivity for K^+ over Na^+ occurs. KcsA (Fig. 1D) has high sequence similarity in this region with the eukaryotic K^+ channels described above (Fig. 1A–C), and consequently provides practical insight into the pores of all homologous K^+ channels. In addition to there being a high-resolution crystal structure available [10,11], KcsA is a well-characterized K^+ channel with both high K^+ conductance and high selectivity against Na^+ ions [12–15, 16]. KcsA has only two transmembrane helices, similar to the Kir channels, and a cytoplasmic domain of unknown function. There are high resolution structures of KcsA in several conformations [11,17], with different ions in the pore [18–20], and in complex with different blockers and antibodies [11,21–23]. This permits thorough investigations of mechanistic aspects of gating and permeation in potassium channels with both theory and experiments.

1.1. Early studies of K^+ channels

In the early 1950s Hodgkin and Huxley released a series of papers in which they describe the laws that control the movements of ions through the surface membrane of giant nerve fibers [24–28]. In doing so they definitively demonstrated that the cell membrane can selectively distinguish between and control the permeation of Na^+ and K^+. One of the earliest theories for this selectivity was conceived by Mullins. He suggested that pores in the membrane were responsible for ion conductance and that selectivity was dependent on size complementarity between the pore and the ion [29]. He further speculated that the pore would remove the outer hydration layers of the ion, leaving a single hydration layer around the ion to interact directly with the interior of the pore [29]. Attempts to pass TEA^+, Na^+, Li^+, or Cs^+ through the squid axon K^+ pore resulted in a drop in conductance suggesting that cations block K^+ permeation through the channel [30–33]. This led Bezanilla and Armstrong to suggest that the potassium channel has a wide intracellular mouth that binds monovalent cations nonselectively followed by an inner tunnel with dimensions specific for a dehydrated K^+ ion, lined by

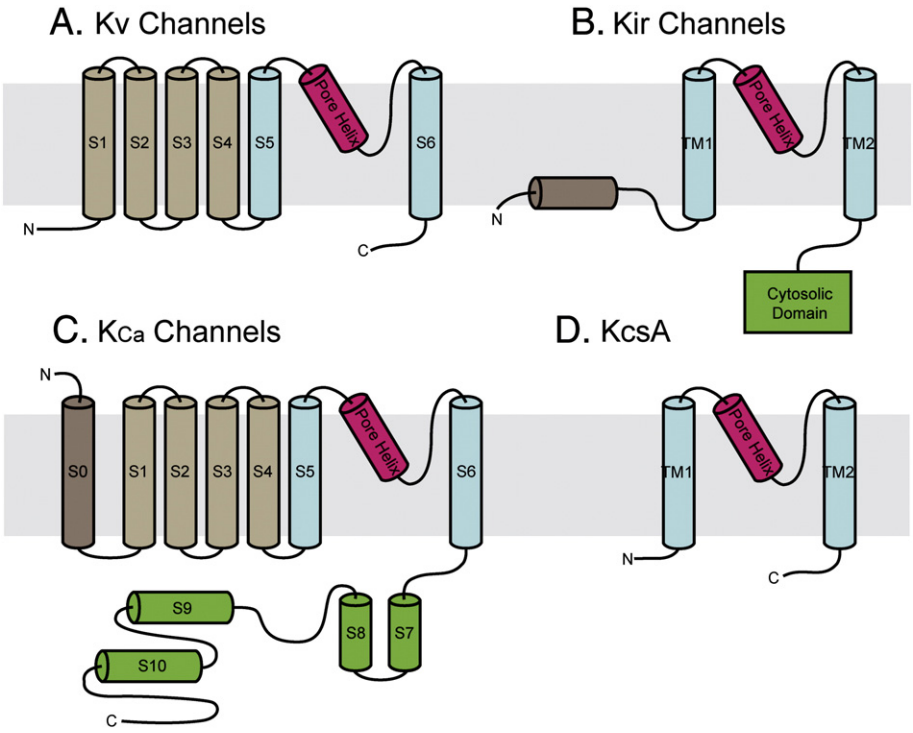

Fig. 1. Topology diagrams of K^+ channels. Transmembrane pore forming helices are cyan, additional transmembrane helices are tan or brown, the helix within the P-loop is pink, and cytosolic helices are green. A. Kv subunits contain six transmembrane helices. Helices S5 and S6 form the pore while helices S3 and S4 function as a voltage sensor. B. Kir subunits contain two transmembrane pore forming helices as well as an additional N-terminal helix and a cytosolic domain that allows the channel to be regulated by different substances. C. K_{Ca} subunits are topologically more similar to Kv channels. $K_{Ca}1.1$ channels have an additional transmembrane helix (S0) for a total of seven. $K_{Ca}2$ and K_{Ca} 3.1 channels have six transmembrane helices like Kv channels, but also contain a cytosolic domain to bind calmodulin. D. Each KcsA subunit is composed of two transmembrane helices connected by a P-loop. These helices extend into the cytosol to form a structural domain that is generally removed to aid crystallization (not shown).

a ring of carbonyl oxygens for solvating K^+ but not ions smaller than K^+ [32]. Further, they speculated that the pore chooses the preferred ion by placing a large energy barrier for small monovalent cations to enter the pore, a process they called selective exclusion. In 1988 Neyton and Miller reassessed this kinetic argument and introduced the idea that the pore chooses its preferred ion via selective binding rather than selective exclusion, a thermodynamic concept in which the ions that permeate bind to specific pore sites with higher affinity than the ions that do not permeate [34,35]. At the same time, they identified 4 different ion-binding sites inside the pore [34,35], reinforcing earlier hypotheses about the single-file multi-ion nature of K^+ channels [36,37].

1.2. The selectivity filter

Cloning of the potassium channel gene [38] allowed the identification of the amino acid composition of the selectivity filter region, TTVGYGD (where only the glycines are strictly conserved), called the signature sequence of potassium channels (Fig. 2) [39]. This signature sequence is found in almost all known potassium channels, with some notable exceptions being Kir 6, EAG, and two-pore-domain K^+ channels that contain a Y to F substitution [40,41]. Systematic mutations of residues in and immediately adjacent to this signature sequence in the voltage gated channel Shaker revealed that specific substitutions can reduce the selectivity of the channel [39]; however, without the structure it was impossible to draw further conclusions about the mechanism of this selectivity change. Also, as demonstrated in a recent study in which the authors failed to increase K^+ selectivity in an HCN4 channel by changing the selectivity filter to match the signature sequence [42], there are clearly other parts of the channel involved in conferring selectivity, such as in Kir3.2 where K^+ selectivity in an unselective mutant was restored by addition of negative charge to the central cavity [43,44].

2. K^+ channels have different selectivities for monovalent cations

A number of cations are able to permeate through K^+ channels. Relative permeabilities are usually determined by calculation of a permeability ratio from reversal potential measurements or from single-channel current–voltage relationships [45]. Early on, it was found that permeability ratios followed the sequence: $P_{Tl}>P_K>P_{Rb}>P_{NH_4}$ in potassium channels from frog nerve fibers, while permeation by Li^+, Na^+, Cs^+, methylamine, guanidine, hydrazine, and hydroxylamine was undetectable [46]. Generally, the permeabilities of K^+, Rb^+, Tl^+, and NH_4^+ are measurable, with small variations across different K^+ channels, while Li^+ and Na^+ do not permeate K^+ channels [47]. However, K^+ channels behave differently with respect to Na^+ depending on whether K^+ is present or not. Some K^+ channels allow Na^+ permeation in the absence of K^+, like Kv3 [48], Kv2.1 [49], Kv1.5 [48,49], KcsA mutants [50], Kir mutants [51], and chimeras and mutants of voltage-gated K^+ channels [52,53], while at the same time preserving high selectivity in the presence of K^+. Other K^+ channels do not allow Na^+ currents in

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kcsa_Sliv_P0A334     LITYPRALWWSVETATTVGYGDLYP--VTLWGRLVAVVVMVAGITSF 103
KvAP_Apernix_Q9YDF8  IKSVFDALWWAVVTATTVGYGDVVP--ATPIGKVIGIAVMLTGISAL 237
MthK_Mthermo_O27564  GESWTVSLYWTFVTIATVGYGDYSP--STPLGMYFTVTLIVLGIGTF  87
MloK1_Mloti_Q98GN8   FGSIPQAMWWAVVTLSTTGYGDTIP--QSFAGRVLAGAVMMSGIGIF 203
shaker_dro_P08510    FKSIPDAFWWAVVTMTTVGYGDMTP--VGVWGKIVGSLCAIAGVLTI 470
kv1.1_human_Q09470   FSSIPDAFWWAVVSMTTVGYGDMYP--VTIGGKIVGSLCAIAGVLTI 400
kv1.2_human_P16389   FPSIPDAFWWAVVSMTTVGYGDMVP--TTIGGKIVGSLCAIAGVLTI 402
kv2.1_human_Q14721   FKSIPASFWWATITMTTVGYGDIYP--KTLLGKIVGGLCCIAGVLVI 405
kv2.2_rabbit_Q95L11  FTSIPASFWWATITMTTVGYGDIYP--KTLLGKIVGGLCCIAGVLVI 409
kv3.1_human_P48547   FKNIPIGFWWAVVTMTTLGYGDMYP--QTWSGMLVGALCALAGVLTI 428
kv3.2_rat_P22462     FKNIPIGFWWAVVTMTTLGYGDMYP--QTWSGMLVGALCALAGVLTI 465
kv4.1_human_Q9NSA2   FTSIPAAFWYTIVTMTTLGYGDMVP--STIAGKIFGSICSLSGVLVI 400
kv4.2_mouse_Q9Z0V2   FTSIPAAFWYTIVTMTTLGYGDMVP--KTIAGKIFGSICSLSGVLVI 398
kv5.1_human_Q9H3M0   FKSIPQSFWWAIITMTTVGYGDIYP--KTTLGKLNAAISFLCGVIAI 398
kv5.1_mouse_Q7TSH7   FKSIPQSFWWAIITMTTVGYGDIYP--KTTLGKLNAAISFLCGVIAI 398
kv6.1_human_Q9UIX4   FTSIPACYWWAVITMTTVGYGDMVP--RSTPGQVVALSSILSGILLM 452
kv6.2_rat_Q9QYU3     FSSVPASYWWAVISMTTVGYGDMVP--RSLPGQVVALSSILSGILLM 410
kv7.1_human_P51787   FGSYADALWWGVVTVTTIGYGDKVP--QTWVGKTIASCFSVFAISFF 340
kv7.2_human_O43526   FDTYADALWWGLITLTTIGYGDKYP--QTWNGRLLAATFTLIGVSFF 305
kir1.1_human_P48048  INGLTSAFLFSLETQVTIGYGFRCVTEQCATAIFLLIFQSILGVIIN 171
kir1.1_rat_P35560    INGMTSAFLFSLETQVTIGYGFRFVTEQCATAIFLLIFQSILGVIIN 164
kir2.1_human_P63252  VNSFTAAFLFSIETQTTIGYGFRCVTDECPIAVFMVVFQSIVGCIID 172
kir2.2_chicken       VNGFVAAFLFSIETQTTIGYGFRCVTEECPLAVFMVVVQSIVGCIID 173
kir3.1_human_P48549  VYNFPSAFLFFIETEATIGYGYRYITDKCPEGIILFLFQSILGSIVD 173
kir4.1_human_P78508  VHTLTGAFLFSLESQTTIGYGFRYISEECPLAIVLLIAQLVLTTILE 158
kir4.1_rat_P49655    VHTLTGAFLFSLESQTTIGYGFRYISEECPLAIVLLIAQLVLTTILE 158
kir5.1_human_Q9NPI9  VHSFTGAFLFSLETQTTIGYGYRCVTEECSVAVLMVILQSILSCIIN 161
kir6.1_human_Q15842  VRSFTSAFLFSIEVQVTIGFGGRMMTEECPLAITVLILQNIVGLIIN 170
kir7.1_human_P35561  VNSFTAAFLFSIETQTTIGYGFRCVTDECPIAVFMVVFQSIVGCIID 172
kir7.1_rat_O70617    ITSFTAAFSFSLETQLTIGYGTMFPSGDCPSAIALLAIQMLLGLMLE 149
kca1.1_human_Q12791  ALTYWECVYLLMVTMSTVGYGDVYA--KTTLGRLFMVFFILGGLAMF 380
kca1.1_chick_Q8AYS8  QLTYWECVYLLMVTMSTVGYGDVYA--KTTLGRLFMVFFILGGLAMF 339
kca2.1_human_Q92952  TSNFLGAMWLISITFLSIGYGDMVP--HTYCGKGVCLLTGIMGAGCT 358
kca2.1_mouse_Q9EQR3  TSNFLGAMWLISITFLSIGYGDMVP--HTYCGKGVCLLTGIMGAGCT 355
kca3.1_human_O15554  TGHLSDTLWLIPITFLTIGYGDVVP--GTMWGKIVCLCTGVMGVCCT 278
kca3.1_mouse_O89109  TGHLTDTLWLIPITFLTIGYGDVVP--GTMWGKIVCLCTGVMGVCCT 276
hcn1_human_O60741    GKQYSYALFKAMSHMLCIGYGAQAP--VSMSDLWITMLSMIVGATCY 386
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Fig. 2. Sequence alignment of representative K^+ channels. All sequences except for chicken Kir2.2 were taken from the Uniprot Knowledgebase and are listed with the appropriate accession number. The Kir2.2 sequence was taken from Protein Data Bank entry 3JYC. Sequence regions highlighted in blue or brown represent residues found in the pore helix or helix TM2/S6 based on the structure of KcsA (PDB ID: 1K4C). Amino acids highlighted in yellow correspond to the signature sequence residues that make up the selectivity filter. Residues represented as red text are shown/predicted to form interactions within the selectivity filter and/or its surrounding scaffolding as depicted in Fig. 3.

the absence of K^+ (for example Shaker and KcsA) and can even become defunct [47,54–56].

$K_{Ca}1.1$ channels also demonstrate high selectivity for K^+ over Na^+ [57,58]. Initial characterizations of selectivity in small conductance Ca^{2+}-activated K^+ channels ($K_{Ca}2$) using reversal potentials found high K^+ selectivity with permeability sequences similar to other K^+ channels and no evidence of Na^+ permeation [6,59,60]. In contrast, a more recent study found relatively large Na^+ and Li^+ conductance in both rat $K_{Ca}2$ and human $K_{Ca}3.1$ with a permeability sequence of $P_K > P_{Rb} >> P_{NH_4} = P_{Cs} \sim P_{Li} \sim P_{Na}$, suggesting that these channels may be less selective than originally believed [61].

Interestingly, despite having a canonical GYG-containing signature sequence for K^+ in the selectivity filter, a permeability sequence of $P_{Tl} > P_K > P_{Rb} \sim P_{Na} >> P_{Li}$ was observed for HCN (hyperpolarization activated and cyclic nucleotide gated) channels [62,63]. Permeation of Na^+ in general is much higher in HCN channels, ranging from 1/3 to 1/5 the permeation of K^+ [64]. In addition Ca^{2+} has also been shown to permeate through certain HCN channels [65,66].

This variability in selectivity raises questions about the molecular identity of the elements that governs these differences among potassium channels with the same signature sequence. KcsA, a prokaryotic potassium channel with a high-resolution structure, a canonical TTVGYGD signature sequence, and permeation properties consistent with those of eukaryotic K^+ channels, is an ideal model to use to begin to understand the mechanism behind these selectivity differences.

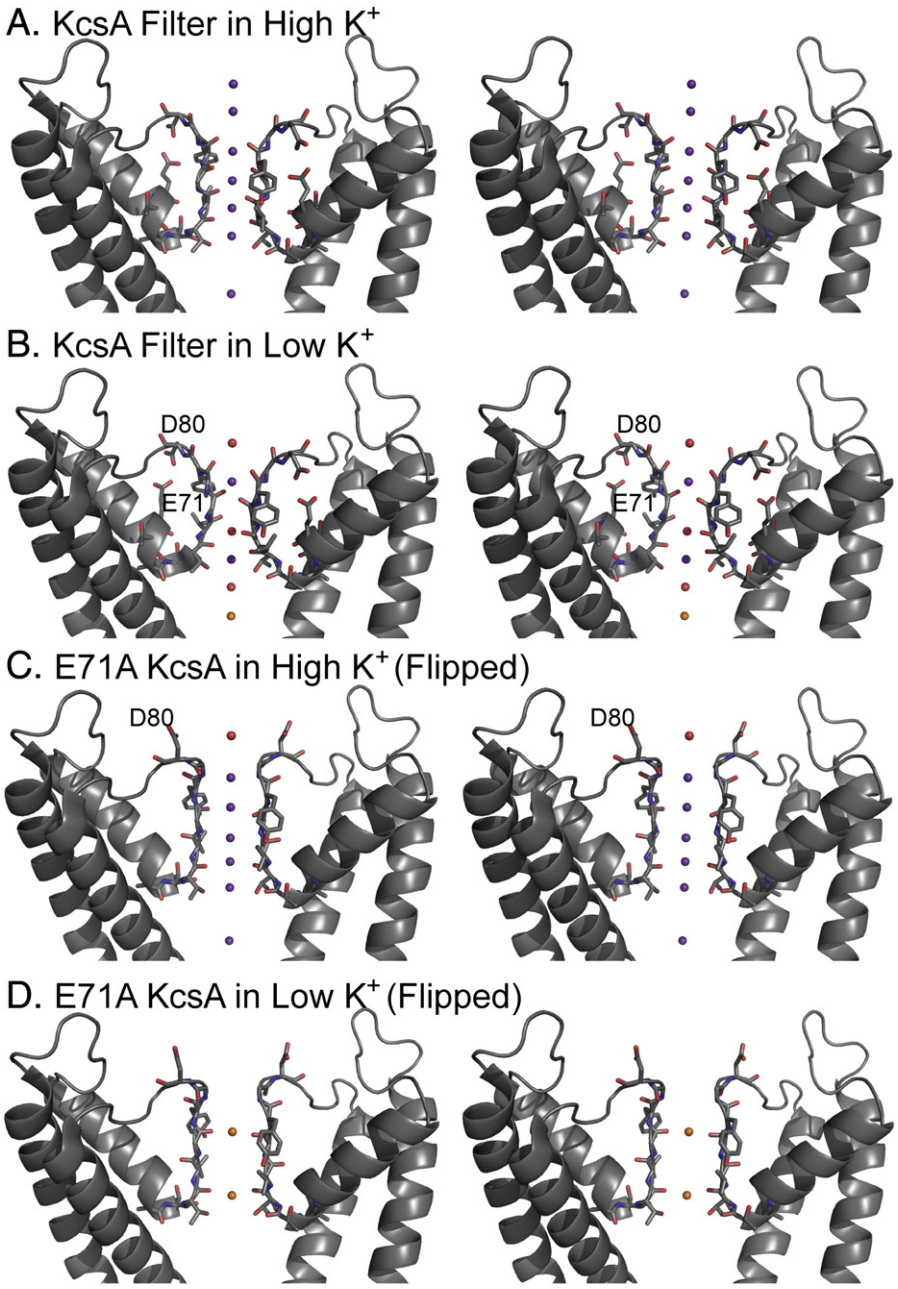

Fig. 3. Stereo diagram depicting different crytallographically observed KcsA selectivity filter conformations. Two subunits have been removed for clarity. Spheres are colored to represent different species: purple for K^+, orange for Na^+, and red for H_2O. A. Active conformation of filter in presence of high K^+ (PDB ID: 1K4C). B. Collapsed filter in the presence of Na^+ and reduced K^+ (PDB ID: 1K4D). C. Filter with E71A mutation in the presence of high K^+. D80 swings out of the selectivity filter and multiple carbonyls within the filter move out of the conduction pathway. Functionally this mutation removes inactivation. D. Filter with E71A mutation in the absence of K^+ and the presence of Na^+. The filter looks very similar to the E71A KcsA channel in the presence of high K^+ suggesting that the E71A mutation removes inactivation by preventing the selectivity filter from collapsing as in Fig. 4B.

3. KcsA, a model K^+ channel

3.1. Function and structure

KcsA was the first potassium channel to have its structure solved. It was identified in the genome of *Streptomyces lividans* and subsequently expressed, purified, reconstituted, and electrophysiologically characterized [12,15,16]. Its sequence suggested that it contained only two transmembrane segments (TM1–2) similar to those of inward rectifying channels, but its P-loop (the loop containing the selectivity filter) sequence and the second, pore-lining transmembrane segment (TM2) shared greater identity with voltage-gated channels (Figs. 1 and 2) [12,16]. It was further shown that the channel was pH gated and only became active in bilayers at a pH below 5 [15,67]. Examination of KcsA selectivity revealed it was similar to that of other potassium channels. These studies included radioactive fluxes ($K^+ > Rb^+ > NH_4^+ >> Na^+ > Li^+$), single channel amplitude measurements in symmetrical solutions ($K^+ > Rb^+ \sim NH_4^+ \sim Tl^+ >> Na^+ > Li^+$), and determination of reversal potentials under bi-ionic or mixed ion solutions ($Tl^+ > K^+ > Rb^+ > NH_4^+, >> Na^+ > Li^+$) [14,15]. Measurements of the K^+/Na^+ permeability ratio as calculated by reversal potentials gave a value greater than 160, whereas measurements of the K^+/Na^+ escape ratio at large depolarized voltages, as determined by relief of block experiments (intracellular Na^+ blocks outward K^+ flux in a voltage dependent way at moderate voltages, while at high voltages, Na^+ escapes to the extracellular side relieving the block and allowing K^+ flux), gave a value of at least 30 [13,14].

The crystallographic structure of KcsA was reported in 1998 [10]. The initial model was refined to a resolution of 3.2 Å and consisted of the entire protein except for the first 22 residues which were not observed in the density and the cytosolic carboxyl terminus which was removed to aid crystallization. The signature sequence described above was located in a loop projected into the center of the channel made up of four symmetric KcsA subunits, such that a narrow pore was formed (Fig. 3A). The carbonyl oxygens of T75, V76, G77, and Y78 and the side chain of T75 were projected into the pore providing 5 rings of oxygens as potential ion binding sites (Fig. 4A). In addition, the carbonyl of G79 was pointing out into the extracellular space above the pore in a manner that strongly suggested it was involved in hydration/dehydration of outgoing/incoming ions.

Three years later, the MacKinnon group published a 2.0 Å structure, achieved by complexing KcsA with an antibody fragment [11]. This high resolution structure contained four single-file K^+ ions between each ring of oxygen atoms such that each K^+ was coordinated by 8 oxygen atoms with bond distances ranging from 2.70 to 3.08 Å (creating 4 binding sites, S1–S4, Fig. 4A), similar to the predictions of Bezanilla and Armstrong [32] and Neyton and Miller [34]. These densities are believed to be the outcome of averaging two distinct occupancy patterns in the 4 binding sites within the selectivity filter (i.e. water–K^+–water–K^+ or K^+–water–K^+–water) [68,69]. In addition, one K^+ ion was modeled in the channel cavity surrounded by 8 water molecules, whereas two K^+ ions were aligned on the external side of the selectivity filter along the central axis of the channel with the outermost one coordinated by four water molecules. The ion coordinated by 8 waters in the cavity was proposed to be representative of a hydrated K^+ in solution with the caveat that this coordination may be influenced by its immediate environment [11,70–72]. Molecular dynamics simulations by Berneche and Roux predicted that a single water molecule was located between each pair of K^+ ions in the selectivity filter and that displacement of ions through the filter was accompanied by fluctuations of V76 and G77 within the filter and E71, D80, and R89 on the extracellular side of the filter [73]. In addition, two potential conduction pathways were observed with very low free energy barriers of 2–3 (knock-on) and 3–4 (vacancy-filling) kcal/mol respectively, indicating that ion conduction through the pore was essentially diffusion limited [74]. Further simulations indicated that selectivity differs throughout the potassium binding sites with site S2 being the most K^+ selective [74–77].

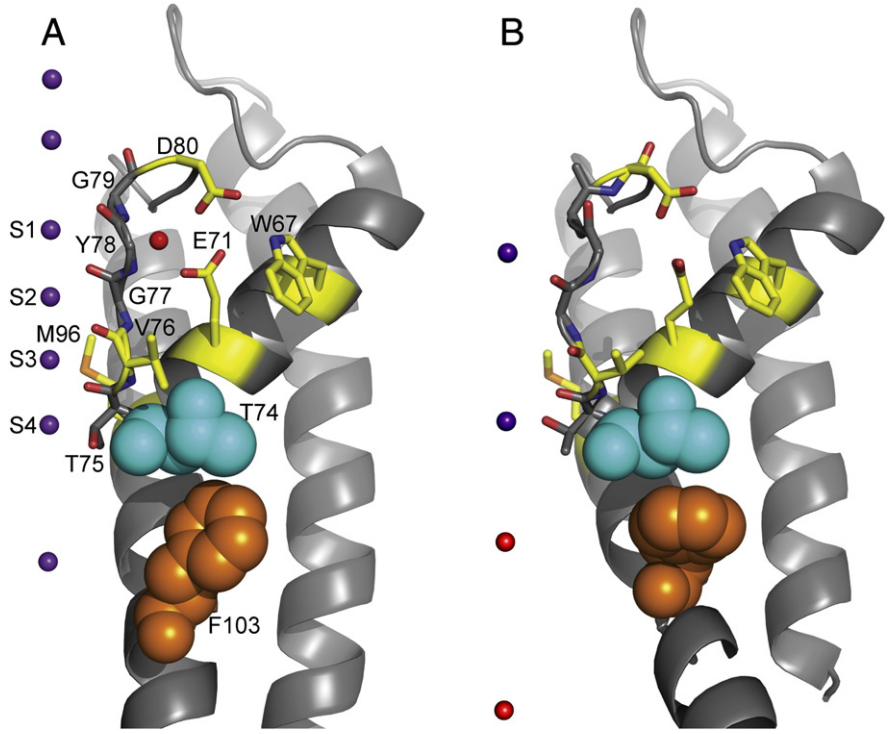

Fig. 4. Residues involved in KcsA inactivation (F103: orange-space filled, T74: cyan-space filled, D80/E71/W67/V76/M96: yellow-stick, K^+: purple sphere, H_2O: red sphere). A. Closed structure of KcsA (PDB ID: 1K4C) with non-collapsed selectivity filter. B. Open structure of KcsA with collapsed selectivity filter (PDB ID: 3F5W). Opening of KcsA via movement of helix TM1 results in contact between F103 (TM1) and T74 (bottom of selectivity filter). In the open structure, the F103 side-chain is shown to rotate to avoid collision with T74. Hydrogen bonds between D80, E71, and W67 have also been linked to inactivation along with interactions between M96 and V76 on a neighboring subunit.

3.2. Selectivity in KcsA

The mechanism by which the selectivity filter allows or prohibits the permeation of ions of relatively similar sizes has been speculated on since the discovery of potassium channels. Knowledge of the selectivity filter structure provided the basis for understanding the mechanism of selectivity at an atomic level. Even so, there are many differing ideas on how the selectivity filter might fulfill this role, and we detail some of these below [78,79].

3.2.1. Close fit model

Mullins suggested back in 1959 that ions move only through pores that fit them closely and that the pores would replace the hydration that the ion had in water [29]. This idea was expanded upon by Bezanilla and Armstrong who predicted that the selectivity filter contained a ring of oxygen atoms rigidly positioned so as to perfectly fit a dehydrated K^+ ion. Na^+ would be selected against because its smaller size would require a higher Coulombic energy to fit in this rigid system than it would in water [32]. Further, they argued that the filter would have to function through "selective exclusion", meaning that the competing ions would have to be selected for by differing binding association rates rather than by differing disassociation rates [32]. The publication of the KcsA structure seemingly supported this model, with the selectivity filter in fact looking very similar to that predicted by Bezanilla and Armstrong [10,32]. Isothermal titration calorimetry (ITC) experiments examining binding of different ions to the selectivity filter of KcsA have shown that ion binding is correlated with ionic radii (volume) and not charge density, further supporting the idea that the protein scaffold around the binding sites is an important determinant in selectivity [18].

3.2.2. Coordination number model

The concept of selectivity via coordination number is similar to that of the close fit model, but the cavity size is of less importance than the number of coordinating ligands [70,79–81]. Varma and Rempe found that in an environment with low dielectric constant, K^+ and Na^+ ions preferred higher coordination numbers of water ligands (8 and 6 vs. 6 and 5) as a result of a decreased electrostatic penalty derived from extracting ligands from their low dielectric solvation phases. Bostick and Brooks used "toy" models to demonstrate that the dipole moments of constrained water molecules could still provide a selective K^+ binding environment and thus were able to substitute for the selectivity filter carbonyls [70]. Thomas et al. also used molecular simulations to show that conformational restraints on the channel ligands played an important role in maintaining selectivity [79].

3.2.3. Field strength model

One of the concerns about the close fit and coordination number models is that the extent of fluctuations in the filter based on the reported crystallographic temperature factors and MD simulations are beyond what would be necessary to adjust for the 0.38 Å difference between the radii of Na^+ and K^+ [82,83]. One theory that eschews the need for a semi-rigid filter is the field strength model, which suggests that the coordinating carbonyl ligands are "liquid-like" and have intrinsic electrostatic properties that regulate ion selectivity [78]. This model is based on the concept of field strength used by Eisenman to calculate the relative selectivity of glass electrodes for various cations [84]. In essence the site selects for ions based on the dipoles of the coordinating ligands [77].

3.2.4. Selectivity: kinetic versus thermodynamic considerations

All of the above selectivity models in K^+ channels are based on thermodynamic considerations. They assess how much more favorable it is to bind a K^+ inside the selectivity filter versus a Na^+, not taking into account the path involved for the ions to get there. The only kinetic consideration comes from Bezanilla and Armstrong who suggested, based on a one-ion pore treatment of K^+ channels, that selectivity for K^+ and against Na^+ from the intracellular side of K^+ channels is due to the much smaller association rate of Na^+ with the filter as compared to K^+ [32]. There is a large energy barrier for Na^+ to enter the filter from the inside as opposed to being practically barrierless for K^+. Recently Thompson et al. have found with a study using single-channel electrophysiology, X-ray crystallography and molecular dynamics simulations that contrary to the previous studies, there are very favorable binding sites for small monovalent cations (Na^+ and Li^+) in the selectivity filter [19]. These sites are at different locations than the K^+ sites. Instead of being "caged" between 8 carbonyl oxygens, they lie within the plane of each ring of 4 carbonyls, allowing better coordination for the smaller ions (previously suggested by Shrivastava et al. [85]). They hypothesized, based on both experiments and molecular dynamics, that one of the determinants for excluding Na^+ from the filter is a large energy barrier formed by K^+ ions occupying their binding sites in the 8-carbonyl cages, and thus preventing Na^+ (or Li^+) from achieving its preferred position in the plane of the carbonyls [19]. There are presumably additional determinants for selectivity as evidenced by the lack of Na^+ conductance in K^+-free wild type KcsA; however, many K^+ channels conduct Na^+ in the absence of K^+ (see Section 2).

4. Selectivity filter inactivation in K^+ channels

Following continuous application of the stimulus, voltage-gated channels undergo a conformational change into a thermodynamically favored nonconducting "inactivated" state, distinct from the closed state. This inactivation occurs in different channels at different rates. Early studies on K^+ channel inactivation in squid neurons and frog skeletal muscle detected a slow inactivation process that differed greatly from the fast inactivation observed in puffer fish neurons and sodium channels in squid neurons [24,86–88]. This particular type of inactivation is voltage dependent and has been called C-type, P-type, or U-type inactivation as it has somewhat different properties in different channels [89–91]. All these processes have in common the fact that they all seem to involve some sort of conformational change at the selectivity filter that renders the channel nonconductive. It should be noted that not all nonconductive selectivity filter states represent inactivation; some ion channels have been suggested to prevent ionic conduction (close) at the selectivity filter instead of the bundle crossing [92–96]. In this review, we will use the term "inactivation" to mean only inactivation events occurring at the selectivity filter. A process similar to this inactivation observed in some voltage-gated K^+ channels was also found in KcsA despite its lack of voltage sensor domains and classical voltage dependence [97,98].

4.1. Inactivation in KcsA

Early simulations of KcsA suggested that the filter became more flexible in the absence of potassium [99,100]. When the high-resolution structure of KcsA was published it was accompanied by a 2.3 Å structure of KcsA in low K^+ [11] (Fig. 3A and B). The low K^+ structure had a distinctly different "collapsed" selectivity filter conformation with the carbonyl of V76 pulled away from the pore and the Cα of G77 twisted inward so as to block the pore [11]. This led to speculation that this might represent the inactive form of KcsA functionally identified later [98,101]. Simulations run on the structure in the absence of K^+ indicated that the structure itself was stable on the timescale of MD simulations and that it was not permeable to K^+, Rb^+, or Na^+ [102]. Recent structures of an open KcsA mutant have shown a correlation between the extent of gate opening and distortion of the selectivity filter [103]. This series of structures has suggested that reorientation of F103 (Figs. 4 and 5A) due to opening of the gate may be responsible for initiating the contacts that eventually lead to the residues that are ultimately responsible for destabilizing the selectivity filter (W67, E71, D80, and

G79) [17,97,104]. Furthermore, it was found that the structure of a non-inactivating KcsA (with a glutamate to alanine mutation at position 71) no longer collapsed in the absence of K^+, suggesting that such collapsed structure could represent an inactivated conformation of the selectivity filter (Fig. 3C and D) [105].

4.2. Kv channels

Early studies on variants of the voltage-gated K^+ channel Shaker revealed that the decline in current through some of these channels required a double-exponential function to properly describe the fit, resulting in two distinct time constants [106,107]. Zagotta and Aldrich also proposed the existence of both fast and slow inactivation processes for Shaker channels patched in *Drosophila* muscle [108]. The amino-terminus of Shaker was shown to be responsible for the fast inactivation process following a series of deletions and point mutations that prevented fast inactivation, and the demonstration that fast inactivation could be reinstated upon addition of the amino-terminal as a peptide [109,110]. As the fast inactivation process was controlled by the amino-terminal it became known as N-type inactivation. The secondary inactivation process was then named C-type inactivation [90]. The same study confirmed that

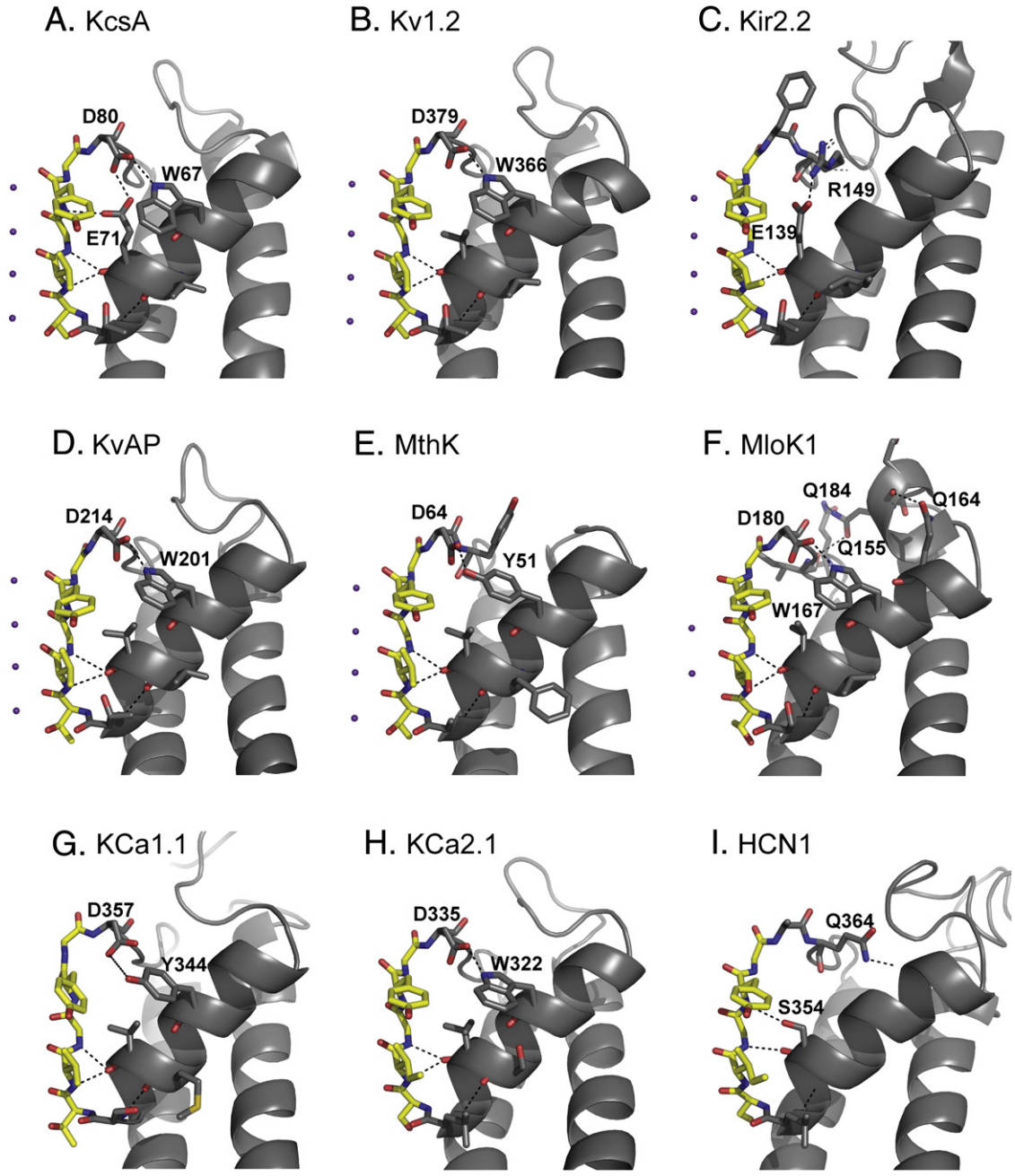

Fig. 5. Cartoon diagrams depicting observed/predicted interactions within the selectivity filters and surrounding scaffolding of several K^+ channels. Selectivity filter residue carbons are colored yellow and K^+ ions are purple spheres. Possible hydrogen bonds are shown as dashed black lines. The first six illustrations were created from the following PDB depositions: A. Kcsa (PDB ID: 1K4C). B. Kv1.2 (PDB ID: 3LUT). C. Kir2.2 (PDB ID: 3JYC). D. KvAP (PDB ID: 1ORQ). E. MthK (PDB ID: 3LDC). F. MloK1 (PDB ID: 3BEH). The final three illustrations: G. K_{Ca}1.1. H. K_{Ca}2.1. I. HCN1, are homology models as there are no experimentally determined structures available. These homology models were generated in ProtMod using KvAP (PDB ID: 2A0L), Kv1.2 (PDB ID: 2A79), and MloK1 (PDB ID: 2zd9) as the respective starting models [149,154,155]. The protein sequence identity and similarity between the initial models and homologous models were 24.6% and 50.9% (KCa1.1), 35.1% and 47.4% (KCa2.1), and 17.5% and 36.8% (HCN1). These values correspond to the 57 residues surrounding the selectivity filter (not the entire protein) and similarity scores were computed using a BLOSUM62 scoring matrix.

C-type inactivation was independent of the presence of the cytoplasmic carboxy-terminal domain.

A number of studies began to suggest that C-type inactivation took place at the external outer mouth of the channel, near the selectivity filter region. Blocking studies in both Shaker and lymphocyte K^+ voltage-gated channels demonstrated that external block by TEA could be used to delay C-type inactivation independently of N-type inactivation [111,112]. TEA blocks K^+ current through Shaker by binding near T449, located external to the selectivity filter, between the pore loop and S6 [113,114] (Fig. 2). Mutation of the same residue in Shaker to a cysteine and addition of Cd^{2+} showed that Cd^{2+} bound to the inactivated state with far greater affinity than to the open or closed states suggesting that the diameter of the pore near the external side of the selectivity filter decreases during inactivation [115]. This point was further strengthened by labeling of cysteines substituted into the surrounding region (M448, T449, and P450) revealing that these residues were more accessible in the inactivated state than in the closed state, and hence closer together [116,117]. Further, it was shown that only during inactivation a disulfide bridge could form between two engineered cysteines at position 448 of two different Shaker channel subunits, suggesting a structural rearrangement in this region of the pore during inactivation [117].

C-type inactivation rates are also dependent on the type of monovalent cations at the external side of the channel [55,56,118–121]. Kv1.3 and Kv1.4 currents are suppressed upon removal of external K^+, but this effect is not observed in Kv1.1 or 1.2 [55,56]. The rate of C-type inactivation in Shaker is decreased with increasing external K^+ [120] and removal of external K^+ lowers the rate at which Kv1.3 recovers from inactivation [122,123]. This argues that the rate of slow inactivation was regulated by the occupancy of an ion binding site at the external mouth of the pore as suggested by Baukrowitz and Yellen [124]. Kiss and Korn demonstrated that this site was in the selectivity filter [119]. Ray and Deutsch also found that recovery from inactivation could be regulated via different intracellular permeant cations [125]. Interestingly the rate of recovery was directly correlated to the rate of permeation of the cation (i.e. $K^+ > NH4^+ > Rb^+ > Cs^+ >> Na^+$) [125]. In agreement with these studies, Ogielska et al. and Panyi et al. found that slow inactivation demonstrated cooperative subunit interactions in both Shaker and lymphocyte Kv1.3 channels [126,127].

Homomeric Kv7.1 (a.k.a KCNQ1 or KvLQT1) channels exhibit a delayed, incomplete inactivation that differs from traditional C-type inactivation in that the inactivation rate is not influenced by external potassium concentrations [128–132]. When in complex with the regulatory subunits of the KCNE family, inactivation is lost entirely [128–130,132,133]. Tryptophan scanning mutagenesis revealed that the KCNQ1 residues 338–340 were likely candidates for interaction with KCNE1 [134]. Mutation of F340 (the residue homologous to F103 in KcsA (Fig. 2), which, as described previously, is crucial for inactivation in the KcsA channel [17]) to a W was shown to reinstate C-type inactivation in the KCNQ1–KCNE1 channel complex [135]. This suggests not only that the mechanism of inactivation is conserved between KcsA and Kv7.1 channels but also that KCNE1 modulates KCNQ1 inactivation through an interaction with F340. Mutation of V310, the selectivity filter residue believed to be closest to the side chain of F340, into alanine or glycine resulted in a similarly enhanced rate of inactivation presumably by removing the interaction with F340 or directly decreasing the stability of the selectivity filter [131].

4.3. Kir channels

Selectivity filter inactivation is generally not observed in Kir channels. One possible exception is Kir1.1. Multiple studies demonstrated that a reduction in the external K^+ concentration could inactivate Kir1.1 and that the rate of inactivation was largely driven by pH [136,137]. Doi et al. found that K^+-dependent inactivation sped up significantly when the intracellular pH was changed from pH 7.5 to pH 6.7. By employing chimeras of Kir1.1 and non-inactivating Kir2.1, they found that this behavior was related to the P-loop region [136]. Schulte et al. further demonstrated that this inactivation was removed by a K80M mutation that eliminated pH gating and found that K^+-dependent inactivation only occurred when the channel was already in a pH inactivated state [137]. They also found that recovery from inactivation could be accomplished with Rb^+ and Cs^+ but not Na^+, further implicating the selectivity filter [137]. A number of mutations in and near the selectivity filter were found based on the sequence of Kir2.1 that prevented K^+-dependent inactivation: L136I V140T, F148C, E151D, and Q152E [137,138]. The mechanistic interpretation of these results was that there are two distinct interacting gates in series; one that was pH dependent (the helix bundle crossing) and one that was dependent on external K^+ concentration (the selectivity filter) [139,140]. Finally, Rapedius et al. found that removal of PIP_2 in the absence of external K^+ led to Kir1.1 inactivation as well [141]. A different form of K^+-dependent inactivation was observed for Kir2.1 channels, induced by hyperpolarization and low external K^+ [142]. Neutralization of Kir2.1 R148, the equivalent of R149 in Kir2.2 (Figs. 2 and 5C) removed this inactivation [142]. The actual mechanism of this inactivation and whether or not it is similar to C-type inactivation is still under study.

4.4. K_{Ca} channels, HCN channels

K_{Ca} channels do not show evidence of slow inactivation. However, the gating of these channels in response to Ca^{2+} is suggested to occur at the selectivity filter, similar to CNG channels [92,94,96,143]. Substituted cysteine accessibility method experiments (SCAM) conducted by Klein et al. suggest that $K_{Ca}3.1$ is incapable of closing at the helix bundle in a manner similar to that described for KcsA or Shaker [143]. This would suggest that gating within KCa3.1 is handled by the selectivity filter or by some other unknown process. SCAM experiments conducted on $K_{Ca}2.2$ also indicate that this channel does not completely close at the helix bundle, and strongly suggests that gating occurs at the selectivity filter [93,94]. Likewise it was shown with a series of bbTBA blockage experiments that $K_{Ca}1.1$ channels are unlikely to close at their bundle crossing as well [96]. Piskorowski and Aldrich show that flickering within the $K_{Ca}1.1$ currents can be increased by replacement with thallium and presumably represent fluctuations of the selectivity filter, showing that although these channels do not inactivate at the selectivity filter, the nature of the permeant ion influences the dynamics of this pore region [95].

5. Coupling of inactivation and selectivity

While all voltage-gated K^+ channels are K^+ selective, some amount of variability has been observed as we noted earlier (see Section 2). Very often changes in selectivity in specific channel mutants appear to be correlated with slow inactivation. Furthermore, during the inactivation process, the filter has been shown to dynamically change its selectivity [53,105,144–146]. The fact that perturbations in the pore that lead to changes in selectivity, also modify the slow inactivation process and vice versa suggests that the two are highly coupled and that they originate in the same pore region. We recently published an article where we proposed a mechanism for how removal of slow inactivation in KcsA channels also affected their selectivity [105]. We will present this proposed mechanism below and we will discuss specific examples in other K^+ channels that show similar coupling between selectivity and inactivation.

5.1. P-loop residues that affect inactivation and/or selectivity

5.1.1. KcsA

Multiple mutations in and near the selectivity filter have led to the creation of mutants in KcsA with varying degrees of inactivation [98]. Inactivation is removed for instance when a glutamate behind the

selectivity filter is changed into an alanine (E71A) [104]. The side chain of E71 had been shown to interact with the side chain of D80, which immediately follows G79 at the other end of the selectivity sequence, in the high-resolution crystal structure of KcsA [11] (Figs. 3A, 4A and 5A). Two distinct crystal structures, both refined to 2.5 Å, were produced from this mutant. One of these was virtually identical to the WT KcsA structure, having only minor deviations in the backbone of the selectivity filter and a slight reorientation of D80 [104]. In contrast, the second structure had a far more extensive rearrangement of its selectivity filter, and the D80 side chain was repositioned so as to interact with an arginine from the co-crystallized antibody fragment (Fig. 3C) [104]. This was called the "flipped" conformation, and it was distinct from the collapsed, "non-conductive" conformation of the filter obtained in low K^+ [11]. The biggest change in the selectivity filter of the flipped structure was that the carbonyl of V76 from each subunit was rotated out of the pore thus distorting two of the K^+ binding sites in the selectivity filter (compare Fig. 3C with A). The other filter carbonyls were slightly distorted such that the coordinated K^+ still occupied the filter, but some of them were shifted from their regular positions. It is not clear at this point whether this is a conformation that would be encountered during normal gating in this variant or whether it is a rarely occurring conformation stabilized by the crystallization conditions.

Despite the drastic change in inactivation induced by the E71A mutation behind the selectivity filter, initial bi-ionic reversal potential measurements did not detect a decrease in K^+/Na^+ selectivity in the E71A mutant [104]. However, a further study by Cheng et al. found evidence of decreased selectivity in E71A KcsA using $^{86}Rb^+$ and $^{22}Na^+$ flux assays as well as experiments measuring relief of Na^+ block through the pore [105]. While WT KcsA was impermeable to Na^+ with or without K^+, Na^+ was far more permeable through E71A KcsA in the absence of K^+, and to some extent in the presence of K^+ [105]. In order to understand the structural causes of this selectivity decrease in the inactivated filter, they obtained the structure of the E71A KcsA in the absence of K^+. This structure did not display the expected collapsed, "non-conductive" conformation encountered in KcsA WT in the absence of K^+, but it looked very similar to the "flipped", presumably conductive structure encountered also in the presence of K^+ [104] (Fig. 3D).

Thus, they hypothesized that there are multiple layers of selectivity in K^+ channels where the first layer, the selectivity provided by the succession of carbonyl oxygen rings from the signature sequence, can be modulated by factors such as modified interactions with the variable sequence behind the filter or excursions to a different filter conformation (such as the collapsed conformation [11]). In the presence of K^+, both KcsA WT and the non-inactivating mutant display strong selectivity against Na^+ likely due to the presence of proper K^+ binding sites (8-carbonyl cages composed of 2 rows of 4-carbonyl rings) in the filter region. However, in the absence of K^+, while the KcsA WT filter collapses and becomes completely non-conductive, the non-inactivating mutant maintains a presumably conductive conformation. Thus, the non-inactivating mutant loses this "second" layer of "steric" selectivity present in the WT, and allows permeation of the ions (in this case Na^+) present in the absence of K^+. What could cause the somewhat reduced selectivity against Na^+ in the presence of K^+ in the non-inactivating mutant? There are at least two possibilities: 1) the "flipped" conformation with the distorted K^+ binding sites (presumably less selective against Na^+ than the "conductive" conformation) is encountered with a certain frequency during normal gating, thus providing less selectivity against Na^+, and 2) the altered electrostatics in the filter due to the E71A mutation that can change barrier heights and well depths for the ions irrespective of whether the "flipped" conformation is encountered during gating.

Furthermore, the fact that the non-inactivating channel no longer collapses in low K^+ strongly indicates that the collapsed conformation is the inactivated state of the channel. Given the low resolution of the structure it is difficult to make strong conclusions about the occupancies of the ion binding sites; however, the only noticeable densities within the filter were directly between the carbonyl oxygens of T75 and those of G77, a binding site more suited for Na^+ than K^+, especially considering the increased Na^+ flux measured through this channel in the absence of K^+ [19,105].

Another manipulation of the KcsA filter that disrupted selectivity and likely affected inactivation was the replacement of G77 with a D-alanine [50,147]. This not only yielded a functional channel, but also prevented the collapse of the selectivity filter in the absence of K^+, as observed in the crystal structure [50,147]. The channel retained its selectivity against Na^+ in the presence of K^+, as expected due to the exact preservation of the K^+ binding sites as well as no major changes in the network behind the filter; however, unlike in WT KcsA, Na^+ currents were observed in the absence of K^+ in the D-A77G mutant, in agreement with the study by Cheng et al. that a non-collapsed structure (irrespective of whether that structure is "flipped" or "conductive") displays cation flux. Although the authors did not ascertain whether the channel still inactivates functionally, the lack of a collapsed structure predicts that this may be the case.

These studies suggest that the same structural elements that induce inactivation in KcsA are also important for maintaining the high selectivity against Na^+ in the absence of K^+. The network of interactions behind the filter modulates both cation selectivity and inactivation in KcsA. We wondered whether this applies to other channels with the same GXG containing signature sequence (where X is tyrosine or phenylalanine). We compiled below a set of examples where changes in selectivity come together with changes in inactivation in other K^+ channels and we tried to correlate these changes with the nature of residues behind the filter.

5.1.2. Other K^+ channels with available structural information

Since the determination of the structure of KcsA, the transmembrane regions of several other K^+ channel structures have been solved. These include the channels KvAP [148,149], MthK [150,151], KirBac1.1 [152], KirBac3.1 [153], and MloK1 [154], which are prokaryotic homologs of voltage-gated channels, Ca-activated channels, inward rectifying channels, and cyclic nucleotide-gated channels respectively. Transmembrane structures of the eukaryotic channels Kv1.2 and Kir2.2 have also been solved [155–158]. Comparison of these structures reveals that the selectivity filter maintains a relatively constant shape, but that the interactions directly behind the selectivity filter differ between channels (Fig. 5). Fig. 5A–F shows the crystallographically determined structures of the selectivity filters of KcsA (PDB ID: 1K4C), human Kv1.2 (PDB ID: 3LUT), chicken Kir2.2 (PDB ID: 3JYC), KvAP (PDB ID: 1ORQ), MthK (PDB ID: 3LDC), and MloK1 (PDB ID: 3BEH) [11,148,151,154,157,158]. The signature sequence residues (yellow) are in similar positions in all six structures. In addition, two carbonyl oxygens at the C-terminal of the pore helix are shown to be within hydrogen bonding distance of the backbone amine groups at one end of the selectivity filter in all six structures (in KcsA these amine groups belong to T74, V76, and G77). In KcsA, an extensive series of hydrogen bonds appears to occur through the side chains of D80, W67, and E71, with E71 also being within hydrogen bonding distance of the amide group of Y78 (Fig. 5A). In addition, the presence of a bulky residue at position 103 (phenylalanine for KcsA) ensures that the message that the bundle-crossing has opened has been transmitted to the selectivity filter [17]. A summary of the residues described here and below is presented in Table 1.

MthK is unique in that the amino acid corresponding to W67 in KcsA is a tyrosine (Y51); however, the tyrosine hydroxyl group is still within hydrogen bonding distance of the KcsA D80 equivalent residue D64 suggesting that bonding is still possible and that tyrosine could replace the tryptophan (Fig. 5E). The presence of a phenylalanine at the position equivalent to F103 in KcsA suggests that MthK may inactivate by a similar mechanism as KcsA. MthK shows an interesting voltage-dependent inactivation proposed to also occur at the selectivity filter [159] and has

Table 1
Equivalent residues in sequence between potassium channels.

Channel	Equivalent residue 1	Equivalent residue 2	Equivalent residue 3	Equivalent residue 4
KcsA	D80	E71	W67	F103
Kv1.2	D379	V370	W366	I402
Kir2.2	R149	E139	L135	D173
KvAP	D214	V205	W201	L237
MthK	D64	V55	Y51	F87
MloK1	D180	V171	W167	F203
$K_{Ca}1.1$	D357	V348	Y344	F380
$K_{Ca}2.1$	D335	I326	W322	T358
HCN1	A363	S354	F350	Y386

Text in red corresponds to residues that are unlikely to serve a similar role to the equivalent residues in KcsA.

high selectivity against Na^+ in the presence of K^+. In the absence of K^+, MthK was shown to conduct Na^+ [151], similar to Kv2.1 channels. In agreement with the findings of Cheng et al. [105], the MthK selectivity filter does not collapse in the absence of K^+ like KcsA [11,151].

KvAP shows voltage-dependent slow inactivation and is strongly selective against Na^+ [160]. KvAP is identical to Kv1.2 (see below) with a presumed hydrogen bond between D214 and W201 (Fig. 5C). In addition, KvAP has a leucine at position 237 (Fig. 2) at a position equivalent with F103 in KcsA. The conservation of these residues suggests that the two channels may inactivate via a similar mechanism. It is not known whether KvAP allows Na^+ current or whether the filter collapses in the absence of K^+, so we cannot comment on how changes in selectivity would affect inactivation.

MloK1 has equivalents to both KcsA D80 and KcsA W67 (D180 and W167) although no equivalent to E71 in KcsA. In addition, MloK1 has a number of glutamine residues Q155, Q164, and Q184 whose side chains are within hydrogen bonding distance of other donor and acceptor groups within the protein chain (Fig. 5F). Furthermore, MloK1 also has a phenylalanine at position 203 equivalent with F103 in KcsA. Conservation of the hydrogen bonding network above the selectivity filter as well as a bulky hydrophobic residue coupling channel opening and closing to the selectivity filter again suggests a similar inactivation mechanism with KcsA. A small single coil helix directly before the pore helix may provide further stabilization in the selectivity filter area. MloK1 currents have not yet been reported. All functional experiments have been performed employing radioactive flux assays [154,161,162].

Of the proteins mentioned here only the structures of KcsA, MthK, and Kv1.2 have been solved at high enough resolution to model water molecules into the selectivity filter. The wild type conductive structure of KcsA contains one water molecule (shown in Fig. 4A) that is within hydrogen bonding distance of the carboxyl groups of E71 and D80 as well as the amides of Y78 and G79 [11]. Both MthK and Kv1.2 contain a water at the same position, as well as a second water near the location of the other E71 (KcsA) carboxyl oxygen (both MthK and Kv1.2 have a valine in the equivalent position of KcsA E71, see Fig. 2) [155,163]. A structure of E71I KcsA also contained two further water molecules, which were in distinct locations from the additional water found in MthK and Kv1.2 [164]. Valiyaveetil and colleagues found that an amide-to-ester change at the linkage between Y78 and G79 resulted in the water not appearing in the subsequent KcsA structure [50]. Finally, Imai and colleagues found in an NMR study that KcsA V76 appeared to interact with a water molecule in the inactivated state but not in the conductive or closed states [165]. Ordered water molecules within the selectivity filter are presumably an important component of the hydrogen bonding network that stabilizes the various channel conductance states.

5.1.3. Kv channels

All Kv channels, including Shaker, have a valine or an isoleucine at the equivalent position to E71 in KcsA, thus lacking the E71–D80 bond that was shown to contribute to inactivation in KcsA (Figs. 2 and 5). However, most of these channels display slow inactivation involving conformational changes at the selectivity filter, suggesting interactions behind the filter probably still play a role in the mechanism of inactivation in these channels. In the Kv1.2 channel, the structurally equivalent residue to KcsA E71 is V370. Still, Kv1.2 displays the remaining hydrogen bond between the W366 and D379 amino acids (corresponding to D80–W67 in KcsA) conserved throughout the voltage gated channel family suggesting an important role for this bond in inactivation and selectivity (Figs. 2 and 5B and Table 1).

One of most studied Kv channels is Shaker. It had been shown that Na^+ could permeate through noninactivated Shaker channels, albeit very poorly and only in the absence of K^+ [52]. However, Starkus et al. demonstrated that inactivated Shaker channels as well as the constitutively inactivated Shaker mutant W434F (homologous to W67 in KcsA) become more permeable to Na^+ and Li^+ in the absence of K^+ [144,145]. Ogielska et al. showed that mutating an alanine in the S6 TM domain of Shaker to a cysteine, reduces selectivity while at the same time slowing inactivation [52,166]. They proposed that the A463C mutation disrupts the K^+ binding sites in the selectivity filter by altering the interaction with V443, which contributes its carbonyl to form a K^+ binding site in the Shaker filter, thus slowing inactivation and reducing selectivity [52,166]. A463 is at the equivalent position to M96 in KcsA, the residue that makes contact with V76 (equivalent to V443 in Shaker) (Fig. 4). M96V yields a KcsA channel that no longer displays a conductive state structurally even at high K^+ concentrations, suggesting a constitutively inactivated state [18].

Kv2.1 channels allow Na^+ permeation in both the absence and presence of K^+ and also inactivate extremely slowly [89], similar to the E71A KcsA mutant discussed earlier [104,105]. Kv 2.1 channels are known to have a larger permeability to Na^+ compared to Shaker-like channels, such as Kv1.3. Chimeras containing the P-loop region of Kv2.1 and the scaffolding Kv1.3 and vice versa demonstrated that neither the P-loop nor the supporting scaffold alone was sufficient to fully transfer selectivity properties [167]. At the same time, the chimera containing the P-loop of Kv1.3 and the scaffolding of Kv2.1 also displayed C-type inactivation similar to Kv1.3 [53,119,127,168].

One of the most compelling studies directly coupling inactivation with changes in selectivity, Kiss et al. demonstrated that certain K^+ channels (Kv2.1, Shaker A463C, and the chimera where the Kv1.3 P-loop was substituted into the equivalent region of Kv2.1) change their selectivity properties dramatically as they progress through the different states of inactivation [53]. Similar effects were also observed in Shaker by Starkus et al. [144,145].

5.1.4. Kir channels

The Kir channels contain a glutamate equivalent to KcsA E71, but do not have either the corresponding aspartate or the tryptophan. Instead, the Kir channels possess a conserved arginine adjacent to the phenylalanine that replaces the aspartate in the TTVGYGD signature sequence (Fig. 2 and Table 1) (one exception to this is Kir7.1 which possesses a methionine instead of the arginine [169]). In the Kir2.2 structure this arginine (R149) interacts with the KcsA E71 equivalent, E139 [158]. The structure of Kir2.2 also reveals a small antiparallel β-sheet involving the three residues behind R149 (C150, V151, and T152) with three residues in the turret region before the pore helix (Figs. 2 and 5C). While these residues are not strictly conserved throughout Kir channels, it is possible that the backbone interactions are.

This salt bridge (E138–R148 in Kir2.1, Fig. 2 and E139–R149 in Kir2.2, Fig. 5C) appears to play an important role in both determining selectivity and inactivation, similar to the E71–D80 interaction described above for KcsA [104,105]. In Kir2.1, single mutations of either the E138 or R148 led to nonfunctioning channels, whereas heterotetramers composed of wild-type and E138D-containing subunits resulted only in gating changes and reduced current without any observed changes in selectivity [170].

However, the reverse double mutation of E138R and R148E gave functioning channels with reduced current and also dramatic loss of selectivity between K^+ and Na^+ [170]. Similar loss of selectivity was observed when the equivalent salt bridge was removed in Kir3.1/3.4 heteromers [51]. In addition, removal of this salt bridge resulted in constitutively open channels that were no longer sensitive to G protein agonists, suggesting that the conformation of the selectivity filter in the presence of salt bridge and absence of agonist (equivalent to a C-type inactivated state in Kv channels) has been destabilized [171]. So, although Kir channels do not display canonical C-type inactivation like some Kv channels, they still undergo some form of gating at the selectivity filter, and disruptions in the selectivity caused by perturbations of the filter conformation via mutations similar to those in KcsA cause changes in this form of gating.

5.1.5. Other channels

Currently there are no structures of eukaryotic calcium-activated (K_{Ca}) or hyperpolarization-activated cyclic nucleotide modulated (HCN) channels. In order to compare the possible interactions behind the selectivity filter with known structures, we have generated homology models of human $K_{Ca}1.1$, $K_{Ca}2.1$, and HCN1 using the ProtMod server [172,173]. Further description of the homology models is provided in the figure legend for Fig. 5. The homology model of $K_{Ca}1.1$ (BK or large conductance Ca^{2+}-activated K^+ channel) shows few interactions between pore loop residues and residues within the selectivity filter (Fig. 5G). The aspartate corresponding to D80 in KcsA is present in Kca1.1 but there are no equivalents in the sequence for E71. The homolog of KcsA W67, Y344 may be able to interact with the aspartate (Fig. 2). $K_{Ca}1$ channels have high selectivity for K^+ over Na^+ ions [57] and do not display slow, C-type inactivation [174]. As far as we know, no mutations have been studied specifically in this channel that change this selectivity or that bring about classical C-type inactivation.

In contrast, the $K_{Ca}2.1$ (SK or small conductance Ca^{2+}-activated K^+ channel) sequence and structure contain conserved equivalents to KcsA D80 and KcsA W67 (D335 and W322 that are shown to interact in our homology model, Fig. 5H). However, these channels lack a bulky residue at the equivalent position with F103 in KcsA. These channels do not inactivate and most investigations report them to have standard high selectivity against Na^+ [6,59,60]. However, $K_{Ca}2$ channels have an altered signature sequence compared to that of most other K^+ channels. Instead of TTVGYGD they contain LSIGYGD (Fig. 2). Mutation of the serine to an alanine (S359A) vastly increased the permeability of Na^+ ($P_{Na}/P_K = 0.4$), whereas the S359T mutation demonstrated permeability similar to that of the wild type channel [175].

HCN channels, despite a GYG signature sequence in the selectivity filter, select very weakly between K^+ and Na^+ (4:1) and do not display inactivation (a notable exception is the sea urchin homolog, spIH, which inactivates very fast in the absence of cAMP and addition of cAMP removes this inactivation) [176]. The HCN1 homology model does not contain equivalents to residues within the KcsA hydrogen-bonding network. It does contain a serine (S354) in an equivalent position to KcsA E71 which could potentially interact with the backbone of the selectivity filter (Fig. 5). The HCN1 model also contains a glutamine (Q364) in a position analogous to the conserved arginine in Kir channels that could potentially interact with the backbone of the pore helix.

In summary, we have observed the following trend with the K^+ channels we examined here: in order for a K^+ channel to inactivate, it needs to have both a network of interactions between the pore helix and the top of the selectivity filter and a bulky amino acid residue at a position that could interact with the bottom of the selectivity filter (equivalent to phenylalanine at position 103 in KcsA). In addition, as described above, we found that in most cases mutations that affect inactivation also appear to affect selectivity. We read this as strong support for the long-held idea that the various types of "C-type inactivation" first described in Kv channels and now throughout K^+ channels, occur by a rearrangement of the selectivity filter.

6. Conclusion

Many of the studies described above have suggested a link between ion selection and selectivity filter inactivation. Most of the manipulations that have been shown to alter these properties have occurred in the selectivity filter and its supporting scaffolding. In this manuscript we have attempted to summarize the current state of knowledge concerning these interactions. We believe that further determination of crystal structures of other K^+ channels such as the eukaryotic Ca^{2+}-activated channels and HCN channels will help map out the important residue interactions that play a role in selectivity and inactivation.

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References

[1] F.H. Yu, W.A. Catterall, The VGL-chanome: a protein superfamily specialized for electrical signaling and ionic homeostasis, Sci. STKE 2004 (2004) re15.
[2] G. Yellen, The voltage-gated potassium channels and their relatives, Nature 419 (2002) 35–42.
[3] H. Hibino, A. Inanobe, K. Furutani, S. Murakami, I. Findlay, Y. Kurachi, Inwardly rectifying potassium channels: their structure, function, and physiological roles, Physiol. Rev. 90 (2010) 291–366.
[4] X.M. Xia, X. Zeng, C.J. Lingle, Multiple regulatory sites in large-conductance calcium-activated potassium channels, Nature 418 (2002) 880–884.
[5] L. Bao, A.M. Rapin, E.C. Holmstrand, D.H. Cox, Elimination of the BK(Ca) channel's high-affinity Ca(2+) sensitivity, J. Gen. Physiol. 120 (2002) 173–189.
[6] B. Lancaster, R.A. Nicoll, D.J. Perkel, Calcium activates two types of potassium channels in rat hippocampal neurons in culture, J. Neurosci. 11 (1991) 23–30.
[7] T.M. Ishii, C. Silvia, B. Hirschberg, C.T. Bond, J.P. Adelman, J. Maylie, A human intermediate conductance calcium-activated potassium channel, Proc. Natl. Acad. Sci. U.S.A. 94 (1997) 11651–11656.
[8] X.M. Xia, B. Fakler, A. Rivard, G. Wayman, T. Johnson-Pais, J.E. Keen, T. Ishii, B. Hirschberg, C.T. Bond, S. Lutsenko, J. Maylie, J.P. Adelman, Mechanism of calcium gating in small-conductance calcium-activated potassium channels, Nature 395 (1998) 503–507.
[9] J.E. Keen, R. Khawaled, D.L. Farrens, T. Neelands, A. Rivard, C.T. Bond, A. Janowsky, B. Fakler, J.P. Adelman, J. Maylie, Domains responsible for constitutive and Ca(2+)-dependent interactions between calmodulin and small conductance Ca(2+)-activated potassium channels, J. Neurosci. 19 (1999) 8830–8838.
[10] D. Doyle, J. Morais Cabral, R. Pfuetzner, A. Kuo, J. Gulbis, S. Cohen, B. Chait, R. MacKinnon, The structure of the potassium channel: molecular basis of K^+ conduction and selectivity, Science 280 (1998) 69–77.
[11] Y. Zhou, J. Morais-Cabral, A. Kaufman, R. Mackinnon, Chemistry of ion coordination and hydration revealed by a K^+ channel-Fab complex at 2. 0 A resolution, Nature 414 (2001) 43–48.
[12] H. Schrempf, O. Schmidt, R. Kummerlen, S. Hinnah, D. Muller, M. Betzler, T. Steinkamp, R. Wagner, A prokaryotic potassium ion channel with two predicted transmembrane segments from *Streptomyces lividans*, EMBO J. 14 (1995) 5170–5178.
[13] C.M. Nimigean, Na^+ block and permeation in a K^+ channel of known structure, J. Gen. Physiol. 120 (2002) 323–335.
[14] M. LeMasurier, L. Heginbotham, C. Miller, KcsA: it's a potassium channel, J. Gen. Physiol. 118 (2001) 303–314.
[15] L.G. Cuello, J.G. Romero, D.M. Cortes, E. Perozo, pH-dependent gating in the *Streptomyces lividans* K^+ channel, Biochemistry 37 (1998) 3229–3236.
[16] L. Heginbotham, L. Kolmakova-Partensky, C. Miller, Functional reconstitution of a prokaryotic K^+ channel, J. Gen. Physiol. 111 (1998) 741–749.
[17] L.G. Cuello, V. Jogini, D.M. Cortes, A.C. Pan, D. G, O. Dalmas, J.F. Cordero-Morales, S. Chakrapani, E. Perozo, Nature Structural basis for the coupling between activation and inactivation gates in K^+ channels, 466 (2010) 272–275.
[18] S.W. Lockless, M. Zhou, R. MacKinnon, Structural and thermodynamic properties of selective ion binding in a K^+ channel, PLoS Biol. 5 (2007) e121.
[19] A.N. Thompson, I. Kim, T.D. Panosian, T.M. Iverson, T.W. Allen, C.M. Nimigean, Mechanism of potassium-channel selectivity revealed by Na(+) and Li(+) binding sites within the KcsA pore, Nat. Struct. Mol. Biol. 16 (2009) 1317–1324.
[20] Y. Zhou, R. MacKinnon, The occupancy of ions in the K^+ selectivity filter: charge balance and coupling of ion binding to a protein conformational change underlie high conduction rates, J. Mol. Biol. 333 (2003) 965–975.
[21] J.D. Faraldo-Gomez, E. Kutluay, V. Jogini, Y. Zhao, L. Heginbotham, B. Roux, Mechanism of intracellular block of the KcsA K^+ channel by tetrabutylammonium: insights from X-ray crystallography, electrophysiology and replica-exchange molecular dynamics simulations, J. Mol. Biol. 365 (2007) 649–662.
[22] S. Yohannan, Y. Hu, Y. Zhou, Crystallographic study of the tetrabutylammonium block to the KcsA K^+ channel, J. Mol. Biol. 366 (2007) 806–814.

[23] M.J. Lenaeus, M. Vamvouka, P.J. Focia, A. Gross, Structural basis of TEA blockade in a model potassium channel, Nat. Struct. Mol. Biol. 12 (2005) 454–459.
[24] A.L. Hodgkin, A.F. Huxley, A quantitative description of membrane current and its application to conduction and excitation in nerve, J. Physiol. 117 (1952) 500–544.
[25] A.L. Hodgkin, A.F. Huxley, The components of membrane conductance in the giant axon of Loligo, J. Physiol. 116 (1952) 473–496.
[26] A.L. Hodgkin, A.F. Huxley, Currents carried by sodium and potassium ions through the membrane of the giant axon of Loligo, J. Physiol. 116 (1952) 449–472.
[27] A.L. Hodgkin, A.F. Huxley, The dual effect of membrane potential on sodium conductance in the giant axon of Loligo, J. Physiol. 116 (1952) 497–506.
[28] A.L. Hodgkin, A.F. Huxley, B. Katz, Measurement of current–voltage relations in the membrane of the giant axon of Loligo, J. Physiol. 116 (1952) 424–448.
[29] L.J. Mullins, An analysis of conductance changes in squid axon, J. Gen. Physiol. 42 (1959) 1013–1035.
[30] W.K. Chandler, H. Meves, Voltage clamp experiments on internally perfused giant axons, J. Physiol. 180 (1965) 788–820.
[31] W.J. Adelman, J.P. Senft, Dynamic asymmetries in the squid axon membrane, J. Gen. Physiol. 51 (1968) 102–114.
[32] F. Bezanilla, C.M. Armstrong, Negative conductance caused by entry of sodium and cesium ions into the potassium channels of squid axons, J. Gen. Physiol. 60 (1972) 588–608.
[33] C. Bergman, Increase of sodium concentration near the inner surface of the nodal membrane, Pflugers Arch. 317 (1970) 287–302.
[34] J. Neyton, C. Miller, Potassium blocks barium permeation through a calcium-activated potassium channel, J. Gen. Physiol. 92 (1988) 549–567.
[35] J. Neyton, C. Miller, Discrete Ba^{2+} block as a probe of ion occupancy and pore structure in the high-conductance Ca^{2+}-activated K^+ channel, J Gen Physiol 92 (1988) 569–586.
[36] W.J. Adelman Jr., R.J. French, Blocking of the squid axon potassium channel by external caesium ions, J. Physiol. 276 (1978) 13–25.
[37] B. Hille, W. Schwarz, Potassium channels as multi-ion single-file pores, J. Gen. Physiol. 72 (1978) 409–442.
[38] D.M. Papazian, T.L. Schwarz, B.L. Tempel, Y.N. Jan, L.Y. Jan, Cloning of genomic and complementary DNA from Shaker, a putative potassium channel gene from *Drosophila*, Science 237 (1987) 749–753.
[39] L. Heginbotham, Z. Lu, T. Abramson, R. MacKinnon, Mutations in the K^+ channel signature sequence, Biophys. J. 66 (1994) 1061–1067.
[40] L. Heginbotham, T. Abramson, R. MacKinnon, A functional connection between the pores of distantly related ion channels as revealed by mutant K^+ channels, Science 258 (1992) 1152–1155.
[41] R.T. Shealy, A.D. Murphy, R. Ramarathnam, E. Jakobsson, S. Subramaniam, Sequence-function analysis of the K^+-selective family of ion channels using a comprehensive alignment and the KcsA channel structure, Biophys. J. 84 (2003) 2929–2942.
[42] N. D'Avanzo, R. Pekhletski, P.H. Backx, P-loop residues critical for selectivity in K channels fail to confer selectivity to rabbit HCN4 channels, PLoS One 4 (2009) e7712.
[43] D. Bichet, M. Grabe, Y.N. Jan, L.Y. Jan, Electrostatic interactions in the channel cavity as an important determinant of potassium channel selectivity, Proc. Natl. Acad. Sci. U.S.A. 103 (2006) 14355–14360.
[44] M. Grabe, D. Bichet, X. Qian, Y.N. Jan, L.Y. Jan, K^+ channel selectivity depends on kinetic as well as thermodynamic factors, Proc. Natl. Acad. Sci. U.S.A. 103 (2006) 14361–14366.
[45] B. Hille, Ion Channels of Excitable Membranes, 3rd ed. Sinauer Associates, Inc, 2001.
[46] B. Hille, Potassium channels in myelinated nerve. Selective permeability to small cations, J. Gen. Physiol. 61 (1973) 669–686.
[47] L. Heginbotham, R. MacKinnon, Conduction properties of the cloned Shaker K^+ channel, Biophys. J. 65 (1993) 2089–2096.
[48] Z. Wang, N.C. Wong, Y. Cheng, S.J. Kehl, D. Fedida, Control of voltage-gated K^+ channel permeability to $NMDG^+$ by a residue at the outer pore, J. Gen. Physiol. 133 (2009) 361–374.
[49] S.J. Korn, S.R. Ikeda, Permeation selectivity by competition in a delayed rectifier potassium channel, Science 269 (1995) 410–412.
[50] F.I. Valiyaveetil, M. Leonetti, T.W. Muir, R. Mackinnon, Ion selectivity in a semisynthetic K^+ channel locked in the conductive conformation, Science 314 (2006) 1004–1007.
[51] K.M. Dibb, T. Rose, S.Y. Makary, T.W. Claydon, D. Enkvetchakul, R. Leach, C.G. Nichols, M.R. Boyett, Molecular basis of ion selectivity, block, and rectification of the inward rectifier Kir3.1/Kir3.4 K(+) channel, J. Biol. Chem. 278 (2003) 49537–49548.
[52] E.M. Ogielska, R.W. Aldrich, A mutation in S6 of Shaker potassium channels decreases the K^+ affinity of an ion binding site revealing ion–ion interactions in the pore, J. Gen. Physiol. 112 (1998) 243–257.
[53] L. Kiss, J. LoTurco, S.J. Korn, Contribution of the selectivity filter to inactivation in potassium channels, Biophys. J. 76 (1999) 253–263.
[54] L.A. Pardo, S.H. Heinemann, H. Terlau, U. Ludewig, C. Lorra, O. Pongs, W. Stuhmer, Extracellular K^+ specifically modulates a rat brain K^+ channel, Proc. Natl. Acad. Sci. U.S.A. 89 (1992) 2466–2470.
[55] F. Gomez-Lagunas, Shaker B K^+ conductance in Na^+ solutions lacking K^+ ions: a remarkably stable non-conducting state produced by membrane depolarizations, J. Physiol. 499 (Pt 1) (1997) 3–15.
[56] H. Jäger, H. Rauer, a.N. Nguyen, J. Aiyar, K.G. Chandy, S. Grissmer, Regulation of mammalian Shaker-related K^+ channels: evidence for non-conducting closed and non-conducting inactivated states, J. Physiol. 506 (Pt 2) (1998) 291–301.
[57] A.L. Blatz, K.L. Magleby, Ion conductance and selectivity of single calcium-activated potassium channels in cultured rat muscle, J. Gen. Physiol. 84 (1984) 1–23.
[58] J.A. Tabcharani, S. Misler, Ca^{2+}-activated K^+ channel in rat pancreatic islet B cells: permeation, gating and blockade by cations, Biochim. Biophys. Acta 982 (1989) 62–72.
[59] M. Kohler, B. Hirschberg, C.T. Bond, J.M. Kinzie, N.V. Marrion, J. Maylie, J.P. Adelman, Small-conductance, calcium-activated potassium channels from mammalian brain, Science 273 (1996) 1709–1714.
[60] Y.B. Park, Ion selectivity and gating of small conductance Ca(2+)-activated K^+ channels in cultured rat adrenal chromaffin cells, J. Physiol. 481 (Pt 3) (1994) 555–570.
[61] N. Shin, H. Soh, S. Chang, D.H. Kim, C.S. Park, Sodium permeability of a cloned small-conductance calcium-activated potassium channel, Biophys. J. 89 (2005) 3111–3119.
[62] A. Ludwig, X. Zong, M. Jeglitsch, F. Hofmann, M. Biel, A family of hyperpolarization-activated mammalian cation channels, Nature 393 (1998) 587–591.
[63] L.P. Wollmuth, B. Hille, Ionic selectivity of Ih channels of rod photoreceptors in tiger salamanders, J. Gen. Physiol. 100 (1992) 749–765.
[64] M. Biel, C. Wahl-Schott, S. Michalakis, X. Zong, Hyperpolarization-activated cation channels: from genes to function, Physiol. Rev. 89 (2009) 847–885.
[65] X. Yu, X.W. Chen, P. Zhou, L. Yao, T. Liu, B. Zhang, Y. Li, H. Zheng, L.H. Zheng, C.X. Zhang, I. Bruce, J.B. Ge, S.Q. Wang, Z.A. Hu, H.G. Yu, Z. Zhou, Calcium influx through If channels in rat ventricular myocytes, Am. J. Physiol. Cell Physiol. 292 (2007) C1147–C1155.
[66] X. Yu, K.L. Duan, C.F. Shang, H.G. Yu, Z. Zhou, Calcium influx through hyperpolarization-activated cation channels (I(h) channels) contributes to activity-evoked neuronal secretion, Proc. Natl. Acad. Sci. U.S.A. 101 (2004) 1051–1056.
[67] L. Heginbotham, M. LeMasurier, L. Kolmakova-Partensky, C. Miller, Single *Streptomyces lividans* K(+) channels: functional asymmetries and sidedness of proton activation, J. Gen. Physiol. 114 (1999) 551–560.
[68] J. Aqvist, V. Luzhkov, Ion permeation mechanism of the potassium channel, Nature 404 (2000) 881–884.
[69] J.H. Morais-Cabral, Y. Zhou, R. MacKinnon, Energetic optimization of ion conduction rate by the K^+ selectivity filter, Nature 414 (2001) 37–42.
[70] D.L. Bostick, C.L. Brooks, Selectivity in K^+ channels is due to topological control of the permeant ion's coordinated state, Proc. Natl. Acad. Sci. U.S.A. 104 (2007) 9260–9265.
[71] S. Varma, S.B. Rempe, Tuning ion coordination architectures to enable selective partitioning, Biophys. J. 93 (2007) 1093–1099.
[72] Y. Zhou, R. MacKinnon, Ion binding affinity in the cavity of the KcsA potassium channel, Biochemistry 43 (2004) 4978–4982.
[73] S. Berneche, B. Roux, Molecular dynamics of the KcsA K(+) channel in a bilayer membrane, Biophys. J. 78 (2000) 2900–2917.
[74] S. Berneche, B. Roux, Energetics of ion conduction through the K^+ channel, Nature 414 (2001) 73–77.
[75] B. Egwolf, B. Roux, Ion selectivity of the KcsA channel: a perspective from multi-ion free energy landscapes, J. Mol. Biol. 401 (2010) 831–842.
[76] V.B. Luzhkov, J. Aqvist, K(+)/Na(+) selectivity of the KcsA potassium channel from microscopic free energy perturbation calculations, Biochim. Biophys. Acta 1548 (2001) 194–202.
[77] S.Y. Noskov, S. Berneche, B. Roux, Control of ion selectivity in potassium channels by electrostatic and dynamic properties of carbonyl ligands, Nature 431 (2004) 830–834.
[78] S.Y. Noskov, B. Roux, Ion selectivity in potassium channels, Biophys. Chem. 124 (2006) 279–291.
[79] M. Thomas, D. Jayatilaka, B. Corry, The predominant role of coordination number in potassium channel selectivity, Biophys. J. 93 (2007) 2635–2643.
[80] S. Varma, S. Rempe, Tuning ion coordination architectures to enable selective partitioning, Biophys. J. 93 (2007) 1093–1099.
[81] H. Yu, S.Y. Noskov, B. Roux, Hydration number, topological control, and ion selectivity, J. Phys. Chem. B 113 (2009) 8725–8730.
[82] D.a. Doyle, The structure of the potassium channel: molecular basis of K^+ conduction and selectivity, Science 280 (1998) 69–77.
[83] S.Y. Noskov, S. Bernèche, B. Roux, Control of ion selectivity in potassium channels by electrostatic and dynamic properties of carbonyl ligands, Nature 431 (2004) 830–834.
[84] G. Eisenman, Cation selective glass electrodes and their mode of operation, Biophys. J. 2 (1962) 259–323.
[85] I.H. Shrivastava, D.P. Tieleman, P.C. Biggin, M.S. Sansom, K(+) versus Na(+) ions in a K channel selectivity filter: a simulation study, Biophys. J. 83 (2002) 633–645.
[86] G. Ehrenstein, D.L. Gilbert, Slow changes of potassium permeability in the squid giant axon, Biophys. J. 6 (1966) 553–566.
[87] S. Nakajima, S. Iwasaki, K. Obata, Delayed rectification and anomalous rectification in frog's skeletal muscle membrane, J. Gen. Physiol. 46 (1962) 97–115.
[88] S. Nakajima, K. Kusano, Behavior of delayed current under voltage clamp in the supramedullary neurons of puffer, J. Gen. Physiol. 49 (1966) 613–628.
[89] M. De Biasi, H.A. Hartmann, J.A. Drewe, M. Taglialatela, A.M. Brown, G.E. Kirsch, Inactivation determined by a single site in K^+ pores, Pflugers Arch. 422 (1993) 354–363.
[90] T. Hoshi, W.N. Zagotta, R.W. Aldrich, Two types of inactivation in Shaker K^+ channels: effects of alterations in the carboxy-terminal region, Neuron 7 (1991) 547–556.
[91] K.G. Klemic, G.E. Kirsch, S.W. Jones, U-type inactivation of Kv3.1 and Shaker potassium channels, Biophys. J. 81 (2001) 814–826.
[92] G.E. Flynn, W.N. Zagotta, Conformational changes in S6 coupled to the opening of cyclic nucleotide-gated channels, Neuron 30 (2001) 689–698.
[93] A. Bruening-Wright, W.S. Lee, J.P. Adelman, J. Maylie, Evidence for a deep pore activation gate in small conductance Ca^{2+}-activated K^+ channels, J. Gen. Physiol. 130 (2007) 601–610.

[94] A. Bruening-Wright, M.A. Schumacher, J.P. Adelman, J. Maylie, Localization of the activation gate for small conductance Ca^{2+}-activated K^+ channels, J. Neurosci. 22 (2002) 6499–6506.
[95] R.A. Piskorowski, R.W. Aldrich, Relationship between pore occupancy and gating in BK potassium channels, J. Gen. Physiol. 127 (2006) 557–576.
[96] C.M. Wilkens, R.W. Aldrich, State-independent block of BK channels by an intracellular quaternary ammonium, J. Gen. Physiol. 128 (2006) 347–364.
[97] J.F. Cordero-Morales, L.G. Cuello, E. Perozo, Voltage-dependent gating at the KcsA selectivity filter, Nat. Struct. Mol. Biol. 13 (2006) 319–322.
[98] J.F. Cordero-Morales, V. Jogini, A. Lewis, V. Vasquez, D.M. Cortes, B. Roux, E. Perozo, Molecular driving forces determining potassium channel slow inactivation, Nat. Struct. Mol. Biol. 14 (2007) 1062–1069.
[99] L. Guidoni, V. Torre, P. Carloni, Potassium and sodium binding to the outer mouth of the K^+ channel, Biochemistry 38 (1999) 8599–8604.
[100] I.H. Shrivastava, M.S. Sansom, Simulations of ion permeation through a potassium channel: molecular dynamics of KcsA in a phospholipid bilayer, Biophys. J. 78 (2000) 557–570.
[101] L. Gao, X. Mi, V. Paajanen, K. Wang, Z. Fan, Activation-coupled inactivation in the bacterial potassium channel KcsA, Proc. Natl. Acad. Sci. U.S.A. 102 (2005) 17630–17635.
[102] C. Domene, S. Furini, Dynamics, energetics, and selectivity of the low-K^+ KcsA channel structure, J. Mol. Biol. 389 (2009) 637–645.
[103] L.G. Cuello, V. Jogini, D.M. Cortes, A.C. Pan, D.G. Gagnon, O. Dalmas, J.F. Cordero-Morales, S. Chakrapani, B. Roux, E. Perozo, Structural basis for the coupling between activation and inactivation gates in K(+) channels, Nature 466 (2010) 272–275.
[104] J.F. Cordero-Morales, L.G. Cuello, Y.X. Zhao, V. Jogini, D.M. Cortes, B. Roux, E. Perozo, Molecular determinants of gating at the potassium-channel selectivity filter, Nat. Struct. Mol. Biol. 13 (2006) 311–318.
[105] W.W.L. Cheng, J.G. McCoy, A.N. Thompson, C.G. Nichols, C.M. Nimigean, Mechanism for selectivity-inactivation coupling in KcsA potassium channels, Proc Natl Acad Sci U S A. 108 (2011) 5272–5277.
[106] L.C. Timpe, T.L. Schwarz, B.L. Tempel, D.M. Papazian, Y.N. Jan, L.Y. Jan, Expression of functional potassium channels from Shaker cDNA in *Xenopus* oocytes, Nature 331 (1988) 143–145.
[107] L.E. Iverson, B. Rudy, The role of the divergent amino and carboxyl domains on the inactivation properties of potassium channels derived from the Shaker gene of *Drosophila*, J. Neurosci. 10 (1990) 2903–2916.
[108] W.N. Zagotta, R.W. Aldrich, Voltage-dependent gating of Shaker A-type potassium channels in *Drosophila* muscle, J. Gen. Physiol. 95 (1990) 29–60.
[109] T. Hoshi, W.N. Zagotta, R.W. Aldrich, Biophysical and molecular mechanisms of Shaker potassium channel inactivation, Science (New York, N.Y.) 250 (1990) 533–538.
[110] W.N. Zagotta, T. Hoshi, R.W. Aldrich, Restoration of inactivation in mutants of Shaker potassium channels by a peptide derived from ShB, Science 250 (1990) 568–571.
[111] K.L. Choi, R.W. Aldrich, G. Yellen, Tetraethylammonium blockade distinguishes two inactivation mechanisms in voltage-activated K^+ channels, Proc. Natl. Acad. Sci. U.S.A. 88 (1991) 5092–5095.
[112] S. Grissmer, M. Cahalan, TEA prevents inactivation while blocking open K^+ channels in human T lymphocytes, Biophys. J. 55 (1989) 203–206.
[113] R. MacKinnon, G. Yellen, Mutations affecting TEA blockade and ion permeation in voltage-activated K^+ channels, Science 250 (1990) 276–279.
[114] L. Heginbotham, R. MacKinnon, The aromatic binding site for tetraethylammonium ion on potassium channels, Neuron 8 (1992) 483–491.
[115] G. Yellen, D. Sodickson, T.Y. Chen, M.E. Jurman, An engineered cysteine in the external mouth of a K^+ channel allows inactivation to be modulated by metal binding, Biophys. J. 66 (1994) 1068–1075.
[116] M. Holmgren, M.E. Jurman, G. Yellen, N-type inactivation and the S4–S5 region of the Shaker K^+ channel, J. Gen. Physiol. 108 (1996) 195–206.
[117] Y. Liu, M.E. Jurman, G. Yellen, Dynamic rearrangement of the outer mouth of a K^+ channel during gating, Neuron 16 (1996) 859–867.
[118] T. Baukrowitz, G. Yellen, Modulation of K^+ current by frequency and external [K+]: a tale of two inactivation mechanisms, Neuron 15 (1995) 951–960.
[119] L. Kiss, S.J. Korn, Modulation of C-type inactivation by K^+ at the potassium channel selectivity filter, Biophys. J. 74 (1998) 1840–1849.
[120] J. Lopez-Barneo, T. Hoshi, S.H. Heinemann, R.W. Aldrich, Effects of external cations and mutations in the pore region on C-type inactivation of Shaker potassium channels, Recept. Channels 1 (1993) 61–71.
[121] S. Marom, I.B. Levitan, State-dependent inactivation of the Kv3 potassium channel, Biophys. J. 67 (1994) 579–589.
[122] D.I. Levy, C. Deutsch, A voltage-dependent role for K^+ in recovery from C-type inactivation, Biophys. J. 71 (1996) 3157–3166.
[123] D.I. Levy, C. Deutsch, Recovery from C-type inactivation is modulated by extracellular potassium, Biophys. J. 70 (1996) 798–805.
[124] T. Baukrowitz, G. Yellen, Use-dependent blockers and exit rate of the last ion from the multi-ion pore of a K^+ channel, Science 271 (1996) 653–656.
[125] E.C. Ray, C. Deutsch, A trapped intracellular cation modulates K^+ channel recovery from slow inactivation, J. Gen. Physiol. 128 (2006) 203–217.
[126] E.M. Ogielska, W.N. Zagotta, T. Hoshi, S.H. Heinemann, J. Haab, R.W. Aldrich, Cooperative subunit interactions in C-type inactivation of K channels, Biophys. J. 69 (1995) 2449–2457.
[127] G. Panyi, Z. Sheng, C. Deutsch, C-type inactivation of a voltage-gated K^+ channel occurs by a cooperative mechanism, Biophys. J. 69 (1995) 896–903.
[128] J. Barhanin, F. Lesage, E. Guillemare, M. Fink, M. Lazdunski, G. Romey, K(V)LQT1 and lsK (minK) proteins associate to form the I(Ks) cardiac potassium current, Nature 384 (1996) 78–80.
[129] M. Pusch, R. Magrassi, B. Wollnik, F. Conti, Activation and inactivation of homomeric KvLQT1 potassium channels, Biophys. J. 75 (1998) 785–792.
[130] M.C. Sanguinetti, M.E. Curran, A. Zou, J. Shen, P.S. Spector, D.L. Atkinson, M.T. Keating, Coassembly of K(V)LQT1 and minK (IsK) proteins to form cardiac I (Ks) potassium channel, Nature 384 (1996) 80–83.
[131] G. Seebohm, P. Westenskow, F. Lang, M.C. Sanguinetti, Mutation of colocalized residues of the pore helix and transmembrane segments S5 and S6 disrupt deactivation and modify inactivation of KCNQ1 K^+ channels, J. Physiol. 563 (2005) 359–368.
[132] M. Tristani-Firouzi, M.C. Sanguinetti, Voltage-dependent inactivation of the human K^+ channel KvLQT1 is eliminated by association with minimal K^+ channel (minK) subunits, J. Physiol. 510 (Pt 1) (1998) 37–45.
[133] G. Seebohm, M.C. Sanguinetti, M. Pusch, Tight coupling of rubidium conductance and inactivation in human KCNQ1 potassium channels, J. Physiol. 552 (2003) 369–378.
[134] G. Panaghie, K.K. Tai, G.W. Abbott, Interaction of KCNE subunits with the KCNQ1 K^+ channel pore, J. Physiol. 570 (2006) 455–467.
[135] G. Panaghie, K. Purtell, K.K. Tai, G.W. Abbott, Voltage-dependent C-type inactivation in a constitutively open K^+ channel, Biophys. J. 95 (2008) 2759–2778.
[136] T. Doi, B. Fakler, J.H. Schultz, U. Schulte, U. Brandle, S. Weidemann, H.P. Zenner, F. Lang, J.P. Ruppersberg, Extracellular K^+ and intracellular pH allosterically regulate renal Kir1.1 channels, J. Biol. Chem. 271 (1996) 17261–17266.
[137] U. Schulte, S. Weidemann, J. Ludwig, J. Ruppersberg, B. Fakler, K(+)-dependent gating of K(ir)1.1 channels is linked to pH gating through a conformational change in the pore, J. Physiol. 534 (2001) 49–58.
[138] H. Sackin, S. Syn, L.G. Palmer, H. Choe, D.E. Walters, Regulation of ROMK by extracellular cations, Biophys. J. 80 (2001) 683–697.
[139] H. Sackin, A. Vasilyev, L.G. Palmer, M. Krambis, Permeant cations and blockers modulate pH gating of ROMK channels, Biophys. J. 84 (2003) 910–921.
[140] A. Dahlmann, M. Li, Z. Gao, D. McGarrigle, H. Sackin, L.G. Palmer, Regulation of Kir channels by intracellular pH and extracellular K(+): mechanisms of coupling, J. Gen. Physiol. 123 (2004) 441–454.
[141] M. Rapedius, P.W. Fowler, L. Shang, M.S. Sansom, S.J. Tucker, T. Baukrowitz, H bonding at the helix-bundle crossing controls gating in Kir potassium channels, Neuron 55 (2007) 602–614.
[142] R.C. Shieh, Mechanisms for the time-dependent decay of inward currents through cloned Kir2.1 channels expressed in *Xenopus* oocytes, J. Physiol. 526 (Pt 2) (2000) 241–252.
[143] H. Klein, L. Garneau, U. Banderali, M. Simoes, L. Parent, R. Sauve, Structural determinants of the closed KCa3.1 channel pore in relation to channel gating: results from a substituted cysteine accessibility analysis, J. Gen. Physiol. 129 (2007) 299–315.
[144] J.G. Starkus, L. Kuschel, M.D. Rayner, S.H. Heinemann, Macroscopic Na^+ currents in the "nonconducting" Shaker potassium channel mutant W434F, J. Gen. Physiol. 112 (1998) 85–93.
[145] J.G. Starkus, L. Kuschel, M.D. Rayner, S.H. Heinemann, Ion conduction through C-type inactivated Shaker channels, J. Gen. Physiol. 110 (1997) 539–550.
[146] Y. Yang, Y. Yan, F.J. Sigworth, How does the W434F mutation block current in Shaker potassium channels? J. Gen. Physiol. 109 (1997) 779–789.
[147] F.I. Valiyaveetil, M. Sekedat, R. Mackinnon, T.W. Muir, Glycine as a D-amino acid surrogate in the K(+)-selectivity filter, Proc. Natl. Acad. Sci. U.S.A. 101 (2004) 17045–17049.
[148] Y. Jiang, A. Lee, J. Chen, V. Ruta, M. Cadene, B.T. Chait, R. MacKinnon, X-ray structure of a voltage-dependent K^+ channel, Nature 423 (2003) 33–41.
[149] S.Y. Lee, A. Lee, J. Chen, R. MacKinnon, Structure of the KvAP voltage-dependent K^+ channel and its dependence on the lipid membrane, Proc. Natl. Acad. Sci. U.S.A. 102 (2005) 15441–15446.
[150] Y. Jiang, A. Lee, J. Chen, M. Cadene, B.T. Chait, R. MacKinnon, The open pore conformation of potassium channels, Nature 417 (2002) 523–526.
[151] S. Ye, Y. Li, Y. Jiang, Novel insights into K^+ selectivity from high-resolution structures of an open K^+ channel pore, Nat. Struct. Mol. Biol. 17 (2010) 1019–1023.
[152] A. Kuo, J.M. Gulbis, J.F. Antcliff, T. Rahman, E.D. Lowe, J. Zimmer, J. Cuthbertson, F.M. Ashcroft, T. Ezaki, D.A. Doyle, Crystal structure of the potassium channel KirBac1.1 in the closed state, Science 300 (2003) 1922–1926.
[153] O.B. Clarke, A.T. Caputo, A.P. Hill, J.I. Vandenberg, B.J. Smith, J.M. Gulbis, Domain reorientation and rotation of an intracellular assembly regulate conduction in Kir potassium channels, Cell 141 (2010) 1018–1029.
[154] G.M. Clayton, S. Altieri, L. Heginbotham, V.M. Unger, J.H. Morais-Cabral, Structure of the transmembrane regions of a bacterial cyclic nucleotide-regulated channel, Proc. Natl. Acad. Sci. U.S.A. 105 (2008) 1511–1515.
[155] S.B. Long, E.B. Campbell, R. Mackinnon, Crystal structure of a mammalian voltage-dependent Shaker family K^+ channel, Science 309 (2005) 897–903.
[156] S.B. Long, X. Tao, E.B. Campbell, R. MacKinnon, Atomic structure of a voltage-dependent K^+ channel in a lipid membrane-like environment, Nature 450 (2007) 376–382.
[157] X. Chen, Q. Wang, F. Ni, J. Ma, Structure of the full-length Shaker potassium channel Kv1.2 by normal-mode-based X-ray crystallographic refinement, Proc Natl Acad Sci U S A 107 (2010) 11352–11357.
[158] X. Tao, J. Avalos, J. Chen, R. MacKinnon, Crystal structure of the eukaryotic strong inward-rectifier K^+ channel Kir2.2 at 3.1 A resolution, Science 326 (2009) 1668–1674.
[159] A.S. Thomson, B.S. Rothberg, Voltage-dependent inactivation gating at the selectivity filter of the MthK K^+ channel, J Gen Physiol 136 (2010) 569–579.
[160] V. Ruta, Y. Jiang, A. Lee, J. Chen, R. MacKinnon, Functional analysis of an archaebacterial voltage-dependent K^+ channel, Nature 422 (2003) 180–185.
[161] C.M. Nimigean, T. Shane, C. Miller, A cyclic nucleotide modulated prokaryotic K^+ channel, J. Gen. Physiol. 124 (2004) 203–210.
[162] C.M. Nimigean, M.D. Pagel, Ligand binding and activation in a prokaryotic cyclic nucleotide-modulated channel, J. Mol. Biol. 371 (2007) 1325–1337.

[163] Y. Jiang, A. Lee, J. Chen, M. Cadene, B.T. Chait, R. MacKinnon, Crystal structure and mechanism of a calcium-gated potassium channel, Nature 417 (2002) 515–522.
[164] S. Chakrapani, J.F. Cordero-Morales, V. Jogini, A.C. Pan, D.M. Cortes, B. Roux, E. Perozo, On the structural basis of modal gating behavior in K(+) channels, Nat Struct Mol Biol 18 (2011) 67–74.
[165] S. Imai, M. Osawa, K. Takeuchi, I. Shimada, Structural basis underlying the dual gate properties of KcsA, Proc Natl Acad Sci U S A 107 (2010) 6216–6221.
[166] E.M. Ogielska, R.W. Aldrich, Functional consequences of a decreased potassium affinity in a potassium channel pore. Ion interactions and C-type inactivation, J. Gen. Physiol. 113 (1999) 347–358.
[167] D. Immke, L. Kiss, J. LoTurco, S.J. Korn, Influence of non-P region domains on selectivity filter properties in voltage-gated K^+ channels, Recept. Channels 6 (1998) 179–188.
[168] L. Kiss, D. Immke, J. LoTurco, S.J. Korn, The interaction of Na^+ and K^+ in voltage-gated potassium channels. Evidence for cation binding sites of different affinity, J. Gen. Physiol. 111 (1998) 195–206.
[169] F. Doring, C. Derst, E. Wischmeyer, C. Karschin, R. Schneggenburger, J. Daut, A. Karschin, The epithelial inward rectifier channel Kir7.1 displays unusual K^+ permeation properties, J. Neurosci. 18 (1998) 8625–8636.
[170] J. Yang, M. Yu, Y.N. Jan, L.Y. Jan, Stabilization of ion selectivity filter by pore loop ion pairs in an inwardly rectifying potassium channel, Proc. Natl. Acad. Sci. U.S.A. 94 (1997) 1568–1572.
[171] T.W. Claydon, S.Y. Makary, K.M. Dibb, M.R. Boyett, The selectivity filter may act as the agonist-activated gate in the G protein-activated Kir3.1/Kir3.4 K^+ channel, J. Biol. Chem. 278 (2003) 50654–50663.
[172] A. Sali, T.L. Blundell, Comparative protein modelling by satisfaction of spatial restraints, J. Mol. Biol. 234 (1993) 779–815.
[173] L. Jaroszewski, L. Rychlewski, A. Godzik, Improving the quality of twilight-zone alignments, Protein Sci. 9 (2000) 1487–1496.
[174] J.N. Barrett, K.L. Magleby, B.S. Pallotta, Properties of single calcium-activated potassium channels in cultured rat muscle, J. Physiol. 331 (1982) 211–230.
[175] J.P. Johnson Jr., W.N. Zagotta, The carboxyl-terminal region of cyclic nucleotide-modulated channels is a gating ring, not a permeation path, Proc. Natl. Acad. Sci. U.S.A. 102 (2005) 2742–2747.
[176] K.B. Craven, W.N. Zagotta, CNG and HCN channels: two peas, one pod, Annu. Rev. Physiol. 68 (2006) 375–401.