Published in final edited form as:

Curr Opin Struct Biol. 2014 August ; 0: 95–101. doi:10.1016/j.sbi.2014.06.004.

Recent progress on the structure and function of the TrkH/KtrB ion channel

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Abstract

Members of the Superfamily of K⁺ Transporters (SKT) are integral membrane proteins that mediate the uptake of ions into non-animal cells. Although these proteins are homologous to the well-characterized K⁺ channel family, relatively little was known about their transport and gating mechanisms until the recent determination of crystal structures for two SKT proteins, TrkH and KtrB. These structures reveal that the SKT proteins are channels, containing a flexible loop in the middle of the permeation pathway that may act as a gate. Two different conformational changes have been observed for the associated gating rings, suggesting different mechanisms of regulation by the binding of nucleotides.

The SKT superfamily of K⁺ transporters: physiology and function

The Superfamily of K⁺ Transporters, or SKT proteins, play a crucial role in ion homeostasis in all kingdoms of life except that of animals. For example, in bacteria, SKT proteins are required for cell growth in media with low K⁺ concentrations [1], while in plants, they confer resistance to high salinity [2,3]. Branches of the SKT superfamily include the KtrB, TrkH, TrkG and KdpA proteins in bacteria, Trk1 and 2 proteins in fungi, and HKT proteins in plants [4].

The SKT proteins are homologous to the tetrameric K⁺ channels, which mediate rapid and selective flux of K⁺ down its electrochemical gradient [5,6]. Early functional studies on TrkH and KtrB suggested that the proteins worked as transporters, not channels, coupling influx of potassium into the cell with the proton-motive force [7] or Na⁺ transport [8], respectively. However, more recent structural and functional studies, especially electrophysiological recordings, showed that these proteins are ion channels that allow ion permeation down its electrochemical gradient [9-11]. Members of the TrkH/TrkG/KtrB subfamily of SKT proteins also form complexes with cytoplasmic soluble proteins that

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Conflict of Interest Statement

The authors have no conflicts of interest to declare.

regulate their activity [12-14]. These regulatory proteins are homologous to the Regulate Conductance of K^+ (RCK) proteins, which either associate with or are part of K^+ channels and affect their gating [13-16].

The crystal structure of TrkH from *Vibrio parahaemolyticus* was the first structure of an SKT protein to be solved [9]. More recently, crystal structures have been solved for TrkH and a closely related homolog KtrB, in complex with their associated RCK domains [10,11]. In this review, we interpret these results for the TrkH and KtrB proteins in light of the extensive knowledge on K^+ channels in terms of ion selectivity and channel gating.

The TrkH/KtrB fold

All members of the SKT family contain four homologous repeats, each of which is distantly related to a simple K^+ channel subunit [5]. A canonical K^+ channel like KcsA contains four identical subunits, each comprising two transmembrane helices connected by a reentrant P-loop (M1-P-M2), which assemble to form a central permeation pathway [17] (Figure 1a). Crystal structures of TrkH and KtrB confirm that an individual protomer of TrkH or KtrB contains four domains each with an M1-P-M2 topology (Figure 1b). TrkH also contains an additional two transmembrane helices (domain 0) at the N-terminus. The four K^+ channel-like domains encircle an ion permeation pathway lined by the pore helices near the extracellular side and the M2 helices near the cytoplasm (Figure 1c).

Interestingly, both TrkH and KtrB proteins exist as stable dimers in the membrane (Figure 1d) and thus have two parallel pores. The extensive dimer interface is formed in large part by the second half of the M2 helix from domain 3 (D3M2b), which is sharply tilted with respect to the membrane norm and extends along the cytoplasmic face of the neighboring subunit before looping back to D4.

Structure of the selectivity filter and implications for ion conduction

The pore has a constricted selectivity filter near the extracellular side formed by the four P-loops from each of the homologous domains. In K^+ channels, this selectivity filter is lined by a stretch of highly conserved residues known as the signature sequences [17,18] (Figure 2a, b). Since the signature sequences on each domain in TrkH and KtrB differ, the selectivity filter does not have four-fold symmetry as do K^+ channels. In fact, only one residue, corresponding to the first glycine residue in the standard signature sequence of TVGYG, is conserved in all four domains. The selectivity filter is also shorter than that of K^+ channels. K^+ channel selectivity filters contain four ion binding sites, each comprising eight backbone carbonyl oxygens arranged to provide octahedral coordination to a bound K^+ [19] (Figure 2c). In the TrkH structure only one K^+ binding site could be confirmed due to the low resolution of available structures, although the filter could accommodate up to three sites [20] (Figure 2d). The site corresponding to S1 in K^+ channels has definitely been lost, likely due to the loss of the second glycine in the signature sequence.

This decrease in apparent K^+ binding sites is notable because studies of K^+ channels have demonstrated that the number of sites is critical both for the high rate of conduction and for selectivity for K^+ over Na^+ . Reducing the number of sites to 3 or 2, in the absence of any

major changes to the remaining sites, suffices to reduce the maximum rate of conduction and render the channel nonselective for K^+ over Na^+ [21,22]. While the maximum ion conduction rate of an SKT protein has not been measured, single-channel recordings of TrkH show permeation of both K^+ and Na^+ , with only a moderate preference for K^+ . This result agrees with previous studies on KtrB [8,23] and fungal Trk homologs [24], which show permeability to both K^+ and Na^+ . Interestingly, some members of the plant HKT subfamily are more selective for Na^+ [25]. Comparison of the selectivity filter residues of Na^+ -selective HKTs with the K^+ -permeating members of the same subfamilies reveals that the difference in selectivity may be attributed to the substitution of the signature sequence glycine in the first homologous domain with serine, which could correspond to the loss of the S2 ion binding site [26].

The role of the intramembrane loop in gating

Single-channel recordings of the TrkH-TrkA complex revealed its unique gating behaviors. The two pores in the dimer have equal conductance and can open independently, but exhibit high co-operativity, as channel openings are tightly clustered into bursts [10]. In K^+ channels, rapid gating is achieved by a constriction formed by the M2 helices near the intracellular entrance to the pore, referred to as the bundle crossing or intracellular gate [27,28]. In both TrkH and KtrB, however, some of the M2 helices are broken into two segments and splayed away from the pore, resulting in the loss of the intracellular constriction. Instead, a constriction is located immediately intracellular to the selectivity filter, where a large non-helical insertion in the middle of transmembrane helix D3M2 extends into the permeation pathway (Figure 2b). This intramembrane loop contains a number of glycines and small polar residues, and appears to be stabilized by salt bridges with charged residues lining the central cavity, including a highly conserved arginine on the fourth domain. The loop connects to the tilted helix D3M2b.

Several lines of evidence point towards a key role for the intramembrane loop in gating. EPR studies on KtrB have shown that the intramembrane loop is solvent-accessible and changes conformation in the presence of K^+ [29], and deletions of residues on the loop in KtrB or point mutations to the conserved arginine in TrkH result in an increase in the rate of uptake through the channel [30]. The intramembrane loop also appears to be required for the sensitivity of TrkH to nucleotide binding to its associated regulatory protein, TrkA, as is discussed in detail in the following section. Together, these observations suggest that the intramembrane loop is involved in gating the channel.

Organization of the RCK gating ring

TrkH is regulated by TrkA, an RCK-domain containing protein that forms a complex with the channel [12,14,31]. RCK domains possess a bilobed architecture, comprising an N-terminal lobe with a Rossman fold connected by a hinge region to a less conserved C-terminal lobe (Figure 3a) [32,33]. Unlike the RCK domains of K^+ channels, the conserved GXGXXG motifs typical of nucleotide binding sites in Rossman folds are preserved in TrkA and KtrA. The activities of both proteins are upregulated by physiological concentrations of

ATP and downregulated by ADP [10,11]. Binding of NADH/NAD⁺ to TrkA has also been reported [10,14], but these compounds do not appear to modulate the activity of the channel.

Crystal structures of both TrkA and KtrA in complex with their associated channels are available. Both proteins form a ring containing eight RCK domains similar to those associated with K⁺ channels, referred to as a gating ring, but because a single TrkA subunit contains two RCK domains in tandem (RCK1 and RCK2), the TrkA gating ring is a tetramer (Figure 3b). The four subunits in TrkA are oriented so that the N lobe from each RCK1 domain (N1) forms an interface with the N1 lobe from one neighboring protomer, whereas the N lobe from each RCK2 (N2) domain forms an interface with the N2 lobe from its other neighboring protomer. This gives the ring an overall "dimer of dimer" symmetry, matching the symmetry of the TrkH dimer. In TrkH, the cytoplasmic loop following the tilted D3M2b helix forms small interfaces with two diagonal N2 domains, while the D1-D2 loops and C-termini on KtrB interact with the N lobes on KtrA (Figure 3c, d).

Conformational states of the gating rings and possible mechanisms of modulation

In order for substrate binding to modulate the function of the channel, the gating ring must undergo substrate-induced conformational changes that can be communicated to structural elements on the channel involved in gating. For RCK gating rings associated with four-fold symmetric channels such as BK channels, MthK and GsuK, current models suggest that a symmetric dilation of the upper layer of RCK domains pulls on the bundle crossing helices to open the intracellular gate [34-38]. This dilation arises from a change in the relative orientation of neighboring RCK domains at the "flexible" interface, equivalent to the intramolecular interface between two RCK domains in a single TrkA subunit, while the interfaces corresponding to the subunit interfaces in TrkA (the "assembly" interfaces) remain fixed. However, given the dimeric assembly and lack of a bundle crossing in the TrkH and KtrB structures, another mechanism is necessary to connect ligand-binding to gating in these proteins. Possible mechanisms based on different conformations in gating ring structures are discussed below.

The structure of TrkA has also been solved both in isolation, and the free TrkA gating ring shows a different conformation than the structure in complex with the channel [10]. In particular, the hinge angle between the two N-lobes at the so-called flexible interfaces decreases (Figure 4a). Unlike the K⁺ channels, two of the four assembly interfaces also change, with the N2 domains moving relative to each other, while the N1-N1 interfaces stay fixed. The overall effect of this conformational change is that the surfaces that interact with the tilted D3M2b helices on the channel move farther away from each other (Figure 4b). If the conformation of the free gating ring is representative of the open structure, then this conformational change immediately suggests a mechanism for how the binding of ATP could be communicated via the D3M2b helix to the intramembrane loop.

In addition to the TrkA structures, there have been multiple structures solved of the free KtrA gating ring [11,39]. These structures suggest that KtrA undergoes a transition from a square, roughly four-fold symmetrical conformation to a rectangular, two-fold symmetric

one when ATP is replaced with ADP (Figure 4). As with TrkA, this conformational change involves two fixed interfaces and two rotating interfaces, as well as a change in the hinge angle at the flexible interfaces. Curiously, this effect is opposite in direction from that proposed for TrkA: the hinge angle decreases in the "open" state when ATP is bound relative to the "closed" state when ADP is bound. Based on this conformational change, an alternate mechanism was proposed for KtrB, in which ATP binding to KtrB results in an outward motion of the D1-D2 loop that opens a gate on the intracellular side of the channel, while the intramembrane loop is opened by another, unknown mechanism. Since the interaction surfaces between the channel and gating ring are different for the TrkH/TrkA complex and the KtrB/KtrA complex, it may be that the two channels are modulated by different conformational changes on the gating ring. It is also possible that the free gating rings have greater flexibility than when in complex with the channels, and the structures of TrkA or KtrA in isolation do not accurately reflect the open state of the complex. Likely, structures of fully open SKT channel in complex with their gating rings will be necessary to resolve this issue.

Acknowledgments

This work was supported by the US National Institutes of Health (R01DK088057, R01GM098878), the American Heart Association (12EIA8850017), and the Cancer Prevention and Research Institute of Texas (R12MZ)

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- TrkH and KtrB contain 4 domains each structurally similar to a K⁺ channel subunit.
- Both proteins are channels with one membrane-spanning pore per protomer.
- Both channels contain an intramembrane loop in the pore that may act as a gate.
- Gating ring structures suggest different possible mechanisms for modulation by ATP.

Figure 1. Topology and structure of SKT proteins

(a) Topology diagram of the K^+ channel KcsA, shown with the extracellular side on top. (b) Topology diagram of TrkH, colored by domain. (c) A protomer of *V. parahaemolyticus* TrkH (3PJZ), viewed from with the plane of the membrane and colored according to the same scheme as in panel b. (d) The TrkH dimer, viewed from the extracellular side. Green spheres correspond to K^+ in the pores.

Figure 2. The permeation pathway and selectivity filter

(a) The signature sequences from each of the four domains of *V. parahaemolyticus* TrkH are shown aligned to the KcsA signature sequence. (b) A TrkH protomer is shown with domains 0, 2 and 4 removed to reveal the permeation pathway. The selectivity filter is marked with a black box, and the intramembrane loop with an arrow. (c) The selectivity filter of KcsA (PDB # 1K4C) is shown with four K^+ binding sites labeled. (d) The selectivity filters of KtrB (4J7C, left) and TrkH (right) are shown with a potassium ion at the confirmed S3 site; other potential sites are labeled according to the nomenclature for KcsA.

Figure 3. Structure of the gating ring

(a) One protomer from the TrkH gating ring (PDB # 4J9U) is shown with the first RCK domain colored orange and the second RCK domain colored teal. The hinge helices are marked with arrows. (b) The TrkH tetramer. Examples of the "assembly" and "flexible" interfaces, in the nomenclature for RCK gating rings, are marked with dashed lines. (c-d) The TrkH-TrkA (c) and KtrB-KtrA (d) complexes are shown with the channels in gray and the gating rings in orange and teal. Structural elements on the channel forming the interface with the gating ring are highlighted in red.

Figure 4. Conformational changes in the TrkA and KtrA gating rings

(a) The "open" and "closed" conformations of a TrkA protomer from the structures of free TrkA (4J9V) and the TrkH-TrkA complex (4J9U) are shown superposed on one N-lobe (top). The ATP- and ADP-bound structures from KtrA (4J90 and 4J91) are shown on bottom. Arrows mark the direction of movement from the presumed "closed" to "open" state in both. The C-lobes are not shown for clarity. (b) The N-lobes only in the TrkA (top) and KtrA (bottom) gating rings are shown in the conformation from the complex or ADP-bound structure (left) and in the isolated or ATP-bound structure (right). The distances between residues that interact with the channel in two diagonal protomers (275 in TrkA, 70 in KtrA) are marked.