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Arranging the elements of the potassium channel: the T1 domain occludes the cytoplasmic face of the channel

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Abstract The voltage-gated potassium channel is currently one of the few membrane proteins where functional roles have been mapped onto specific segments of sequence. Although high-resolution structures of the transmembrane portions of three bacterial potassium channels, the tetramerization domain and the cytoplasmic "ball" are available, their relative spatial arrangement in mammalian channels remains a matter of ongoing debate. Cryo-electron microscopic images of the six transmembrane voltage-gated Kv channel have been reconstructed at up to 18 Å resolution, revealing that the T1 domain tetramerizes and is suspended below the transmembrane segments. However, the resolution of these images is insufficient to reveal the location of the third piece of the puzzle, the inactivating ball domain. We have used the aberrant interactions observed in a series of chimæric channels to establish that an assembled T1 domain restricts access to the cytoplasmic face of the channel, suggesting that the N-terminal "ball and chain" may be confined in the space between the T1 domain and the transmembrane portion of the channel.

Keywords Activation · N-terminus · Potassium channels · Structure · Voltage dependence

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Introduction

The physiology of a neuronal cell is dictated in no small part by the complement of ion channels it expresses, particularly the voltage-gated potassium channels that sculpt the falling phase of the action potential. These are tetrameric proteins, each subunit of which has six membrane-embedded helices and a re-entrant P-loop that lines the aqueous pore. The key elements whose functions have been identified are the inactivation gate, the voltage sensor and the pore lining loop. Rapidly inactivating channels have a ball-like structure at the N-terminus of their cytoplasmic domains, which physically occludes open channels, causing inactivation by the "ball and chain" mechanism (Zagotta et al. 1990). The fourth transmembrane segment (S4) is positively charged and has been shown to move in response to changes in transmembrane electrical potential (for review, see Bezanilla 2000), thus serving as a voltage sensor. The last two helices and the included loop contribute to the lining of the aqueous pore and carry the elements of ionic selectivity (Sather et al. 1994; MacKinnon 1995). Movement of the last transmembrane helices S5 and S6 has been associated with channel opening and closing (Yellen 1998). Between the first transmembrane helix and the N-terminal ball is a segment called the T1 domain, which is responsible for restricting the promiscuity of subunit–subunit interactions in the formation of the functional tetramer (Shen and Pfafinger 1995; Xu et al. 1995).

Functional roles have been mapped onto specific segments of the sequence of K^+ channels by employing a combination of molecular biology and electrophysiology (Yellen 1998). However, a detailed understanding of the mechanism by which these proteins function requires a knowledge of their three-dimensional architecture. The structures of three major segments of the α-subunit of the K^+ channel have been solved at atomic resolution: the central ion-conducting pore in the form of four bacterial potassium channels (Doyle et al. 1998;

Jiang et al. 2002, 2003a; Kuo et al. 2003); the cytoplasmic tetramerization domain T1 (Kreusch et al. 1998; Bixby et al. 1999) and the N-terminal cytoplasmic "ball" structure responsible for rapid N-type inactivation (Antz et al. 1997). The crystal structures of a bacterial voltage-gated K^+ channel, KvAP, that have been solved recently (Jiang et al. 2003a) provide insight into the possible architecture of the membrane-embedded portion of the protein (see Sigworth 2003 for comments). In addition, low-resolution cryo-electron microscopic (cryo-EM) structures of Shaker (25 Å) and of mammalian Kv1.1 channels (18 Å) have been reported (Sokolova et al. 2001; Orlova et al. 2003). The organization of the cytoplasmic structural elements of channel with respect to the membrane-embedded portion is still to be elucidated.

Much of the data mapping function onto structure has been based on domain swaps using Shaker, a *Drosophila* K^+ channel where diversity is brought about by alternative splicing. A common exon in all transcripts encodes a region stretching from the T1 cytoplasmic domain through almost the entire transmembrane region. Mammalian K^+ channel subunits are encoded on separate genes with high, but not absolute, conservation in this region (Stuhmer et al. 1989; Ramaswami et al. 1990). Thus, domain swaps among mammalian K^+ channels can generate novel phenotypes by aberrant interactions not normally observed in nature [see Varshney and Mathew (2003b) for a review on cytosolic control of channel function]. We have generated a chimæric channel, 4N/1, composed of the N-terminal cytoplasmic region of the rapidly inactivating human Kv1.4 and the transmembrane body of the non-inactivating hKv1.1. The 4N/1 chimæra mediates inward currents on hyperpolarization (Chanda et al. 1999b), owing to the aberrant interaction of the two portions of the channel. We have used the appearance of voltage-dependent inward currents to assay the accessibility of the cytoplasmic face of the channel to the ball and chain segment. We show that the presence of an intact T1 domain occludes the transmembrane body of the channel, preventing access to ball and chain peptides in the cytosol. The data suggest that the ball and chain can assemble within the basket formed by the T1 tetramer suspended from the transmembrane embedded portion of the channel.

Methods

Potassium channel cDNAs were cloned from human brain stem and were subcloned into pGEMA (Ramaswami et al. 1990). The 4N/1 chimæra was generated by transplanting the first 210 residues of hKv1.4 onto hKv1.1, as described earlier (Chanda et al. 1999b) and schematically indicated in Fig. 1B. A start site was introduced into hKv1.1 near the chimæra junction to generate the truncated construct Δ78N1 (Chanda et al. 1999b). A construct 4_{BC} (Δ410C4) encoding just the "ball and chain" segment of hKv1.4 and consisting of the first 210 residues was generated and cloned downstream of the T7 promoter in pGEMA (Chanda et al. 1999a) (Fig. 1B). Similarly, The 4N/1T1 and 4Nb/1T1 chimæric channels were constructed by transplanting the first 183 and 39 residues of hKv1.4, respectively, onto 480 transmembrane amino acids of hKv1.1 as described earlier (Varshney and Mathew 2003a) and schematically indicated in insets to Fig. 3A and Fig. 3C. The T1 domain of hKv1.1 starts 20 residues downstream from the junction in these chimæras. All mutagenesis was confirmed by sequencing the constructs on an ABI PRISM 310 automated sequencer. Methods of *Xenopus* oocyte retrieval and their maintenance have been previously described (Ramaswami et al. 1990; Varshney et al. 2002). Capped polyadenylated RNA of all the constructs was generated using T7 RNA polymerase (mMessage mMachine transcription kit, Ambion, USA). 46 nL of transcribed RNA (150–300 ng/μL) was injected per oocyte 48 h prior to recording. RNA injection, two-electrode voltage clamp of *Xenopus* oocytes and data analysis were carried out as described earlier (Ramaswami et al. 1990; Varshney et al. 2002). Voltage-dependent activation and inactivation curves are best fits to the Boltzmann functions, as described previously (Ramaswami et al. 1990).

Results and discussion

A series of chimæric and truncated constructs were generated using parental channels hKv1.1 and hKv1.4 (schematically represented in Fig. 1A). The chimæras 4N/1, 4N/1T1 and 4Nb/1T1 consist of N-terminal regions of hKv1.4 (the "ball and chain") spliced onto the body of hKv1.1. Figure 1B presents an alignment of the sequences of hKv1.1, hKv1.4, 4N/1, 4N/1T1, 4_{BC} and Δ78N1 over the T1 region. The secondary structures taken by amino acid sequence are indicated against the alignment: five stretches of β strand and four of α helices. The third and fourth β strands are deleted in the making of the 4N/1 chimæra. It is conceivable that the T1 region of 4N/1 may tetramerize. However, the domain has 28 charged residues out of 93 and the net charge over the T1 domain changes from +2 to +4 in going from hKv1.1 to the 4N/1 chimæra. This, together with the 10 amino acid deletion in the middle of the chimæric T1, makes it unlikely that it will form a stable tetramer (Minor et al. 2000). Similarly, the T1 residues remaining in 4_{BC} could lead to an association with the truncated T1 in Δ78N1, thus facilitating tetramerization when the two constructs are expressed together. However, given the extent of deletions in both of the constructs, this association appears extremely unlikely. Nonetheless, the data presented here do not directly address this issue.

In contrast, the transplant site in the 4N/1T1 and 4Nb/1T1 chimæras is situated 20 residues upstream of the T1 domain (Varshney and Mathew 2003a), which therefore have intact T1 domains derived entirely from hKv1.1 (Fig. 1B). The truncation construct, Δ78N1, is generated by deleting the N-terminal residues of hKv1.1 at the splice site in the 4N/1 chimæra. Thus, the Δ78N1 construct consists of the hKv1.1 portion of 4N/1 which lacks the N-terminal chain of hKv1.1 and contains a truncated T1 domain (Fig. 1A, B). The complementary truncation construct 4_{BC} encodes the N-terminus region of hKv1.4 that was transplanted onto the transmembrane body of hKv1.1 to generate the 4N/1 chimæra (Fig. 1A, B).

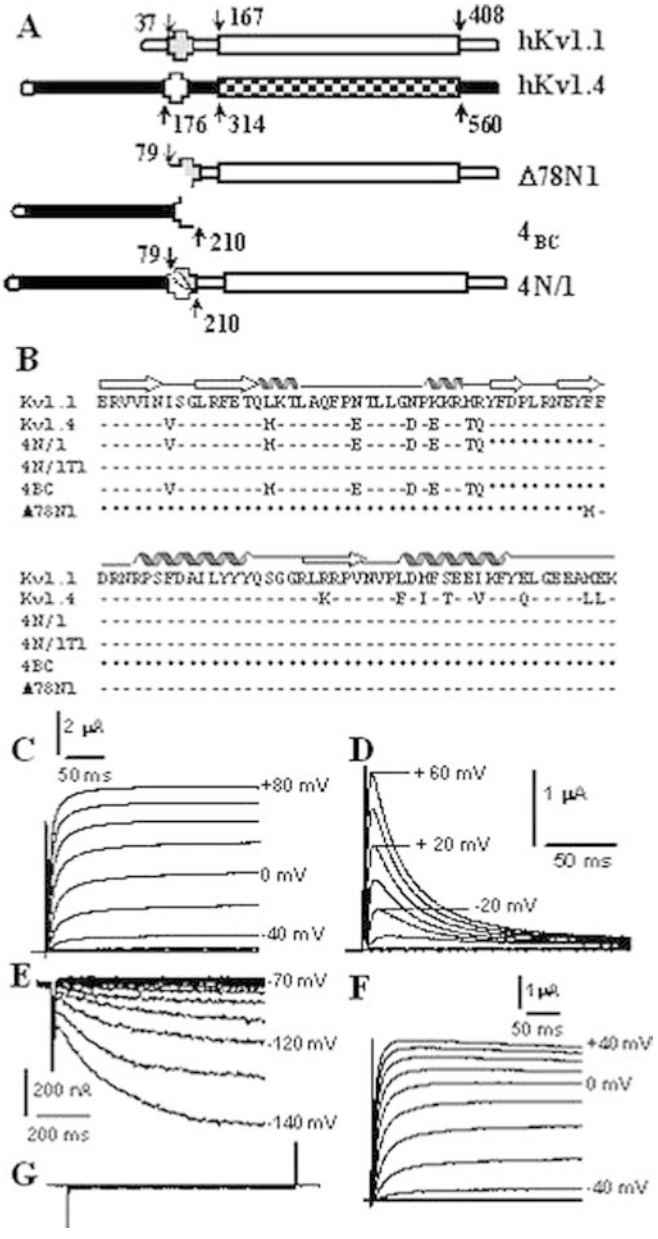

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Fig. 1A–G Chimæric channels derived from two outwardly rectifying channels generate inward currents. Schematic representation of potassium channel constructs. **A** Bar representation for the constructs examined in this study. *Arrows* and *numbers* indicate residues at the junctions or truncations of the channel constructs. For hKv1.1 (*down arrows*) and hKv1.4 (*up arrows*) the arrows indicate the start of T1 domain and also the transmembrane region with residues numbers. Intact T1 domains are depicted with a *solid cross* while a *broken cross* indicates a disrupted T1. **B** Multiple sequence alignment of the T1 region of various channel constructs, done online by the ClustalW (1.82) program at http://www.ebi.a-c.uk/clustalw. The *dashes* denote sequence identity while *stars* symbolize gaps. **C–G** Currents elicited in oocytes expressing these constructs. **C**, **D**, **F** Outward currents elicited on depolarizing oocytes expressing hKv1.1 (**C**), hKv1.4 (**D**), and Δ78N1 (**F**). Oocytes were held at −80 mV and stepped to the indicated potentials following a 1 s prepulse to −120 mV. **E**, **G** Inward currents elicited on hyperpolarizing oocytes expressing 4N/1 (**E**), generated by swapping 210 N-terminal residues of hKv1.4 on to hKv1.1 with a junction within the T1 domain. Oocytes were held at −70 mV and hyperpolarized to potentials indicated against the traces ranging from −70 to −140 mV in −10 mV steps. No such currents were observed (**G**) when the same hyperpolarizing pulse protocol was delivered to oocytes expressing hKv1.1 or hKv1.4 or Δ78N1 in independent experiments

The parental channels mediate outward currents on depolarization, rapidly inactivating currents for hKv1.4 and non-inactivating currents for hKv1.1 (Ramaswami et al. 1990) (Fig. 1C, D). As described previously, the 4N/1 chimæra mediates very little outward current but significant inward currents and functions essentially as an inward rectifier (Chanda et al. 1999b) (Fig. 1E). The shift in operating range is not brought about by deletion of the N-terminal of hKv1.1 since the truncation construct, Δ78N1, exhibits outward currents (Fig. 1F) with the same voltage dependence as full-length hKv1.1 (Chanda et al. 1999b). The parental channels and the truncation mutant, Δ78N1, do not exhibit any currents on membrane hyperpolarization (Fig. 1G).

The conversion of an outward rectifier, hKv1.1, to an inward rectifier, 4N/1, by swapping the cytoplasmic N-terminal of hKv1.1 for the N-terminus of hKv1.4, suggests an interaction between introduced residues from hKv1.4 with the cytoplasmic face of the hKv1.1 channel. Indeed, the co-expression of Δ78N1 and the N-terminal peptide from hKv1.4 (4_{BC}) reconstitutes the inward rectifier (Fig. 2A). The Δ78N1 channel alone fails to open on membrane hyperpolarization and conducts outward currents on depolarization (Fig. 1F, G).

The tetramerization state of the T1 domain is unlikely to directly influence the voltage sensitivity of the channel, as truncation mutants lacking the T1 open over essentially the same voltage range as the wild-type channels (Kobertz and Miller 1999). The shift in operating range observed in the 4N/1 chimera and on co-expressing 4_{BC} with Δ78N1 must arise due to a perturbation of either the voltage sensing apparatus or the machinery that transduces movements of the sensor into channel opening in hKv1.1. The co-expression experiment implicates residues from hKv1.4 present in the 4_{BC} peptide.

The shift in operating range of the Kv1.1 channel could have been brought about either by shifting the gating transition far into the hyperpolarizing regime, or by a fundamental change in the direction of motion of the voltage sensor. In the former case, the channel would have to be kept in an inactivated state at resting membrane potentials and the measured currents would arise from recovery from inactivation rather than a closed-to-open transition (Miller and Aldrich 1996; Smith et al. 1996). The voltage sensor lies close to the cytoplasmic face in the crystal structure of the closed state of the KvAP channel (Jiang et al. 2003b) and would thus be accessible for interaction with the 4_{BC} peptide. The latter scenario is in keeping with reports of gating currents consistent with voltage sensors moving inward in response to hyperpolarization (Mannikko et al. 2002; Latorre 2003; Sesti et al. 2003) and would require an

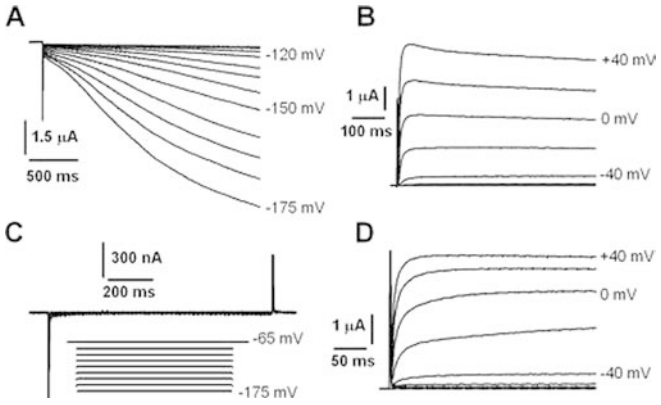

Fig. 2A–D Co-expression of segments derived from outwardly rectifying channels generates inward currents only in absence of an intact T1 domain. Potassium currents elicited in oocytes co-expressing (**A**) Δ78N1 and 4_{BC} and (**C**) full-length hKv1.1 and 4_{BC} on membrane hyperpolarization. Oocytes were held at −65 mV holding potential before stepping to various hyperpolarizing potentials, as shown in the *inset* to **C**. **B**, **D** K^+ outward currents from oocytes co-expressing 4_{BC} with Δ78N1 (**B**) and with full-length hKv1.1 (**D**), using the depolarizing pulse protocol explained in Fig. 1

alternate coupling to movement of the S6 helix. It is not immediately obvious from the KvAP structure as to how such a coupling would be effected.

In the absence of gating current measurements, it is not possible for us to determine which of these gating mechanisms is operating in the 4N/1 chimæra. Irrespective of the mechanism, however, the shift in channel operating range is diagnostic of the ability of the ''ball and chain'' peptide 4_{BC} to perturb either the sensing or the transducing machinery and hence its access to the cytoplasmic face of the transmembrane portion of hKv1.1.

The question of whether the formation of an intact T1 would prevent access was addressed by co-expressing 4_{BC} with full-length hKv1.1. Oocytes co-expressing these constructs exhibit outward currents, with all the characteristics of hKv1.1, on depolarization (Fig. 2D) but no inward currents on hyperpolarization (Fig. 2C). Data in Fig. 2A and Fig. 2B for 4_{BC} co-expressed with Δ78N1, and in Fig. 2C and Fig. 2D for 4_{BC} co-expressed with hKv1.1, are from the same batch of oocytes, indicating that non-expression of 4_{BC} is unlikely to be the reason for the non-observance of inward currents in the latter set. The T1 domain thus occludes the cytoplasmic face of the channel, establishing that the windows lined by the S1–T1 linkers suspending the tetramerized T1 domain below the transmembrane portion of the channel are too small to permit the passage of the 4_{BC} peptide.

Neither 4N/1 nor the inward rectifier reconstituted from its parts (4_{BC} co-expressed with Δ78N1) inactivates rapidly (Fig. 2B) and it could be argued that while the windows are too small for 4_{BC} to pass, the ball alone could possibly enter and cause inactivation. Conversely, if the T1 domain is docked onto the transmembrane segment with ions passing through the T1 pore en route to the K^+ channel pore as suggested by Choe and co-workers (Kreusch et al. 1998; Choe et al. 1999), inactivation could be brought about by the ball binding to the cytoplasmic mouth of the T1 pore. Access of the chain to the transmembrane region need not normally occur in this architecture. Truncation of the native chain does not lead to any significant changes in channel physiology, as seen by comparing Δ78N1 and hKv1.1, suggesting that there are no functional consequences to loss of such an interaction if it occurs in the native channel. In order to address this issue, we constructed the 4N/1T1 chimæra, where the splice site is situated upstream of the T1 domain.

Oocytes expressing the 4N/1T1 chimæra exhibit rapidly inactivating outward currents on depolarization (Fig. 3A). The voltage dependence of the currents is indistinguishable from that of hKv1.1, while the rates and voltage dependence of inactivation are similar to those observed with hKv1.4 (Fig. 3D). The ''ball receptor'' in hKv1.1 is quite accommodating, as peptides corresponding to the inactivating particles from Kv1.4 (Antz et al. 1997), Kv3.4 (Stephens and Robertson 1995) and the beta subunit Kvβ1.1 (Wissmann et al. 1999), all cause rapid inactivation of Kv1.1 channels. Thus, the rapid inactivation seen in 4N/1T1 is indicative of interaction of the ball of hKv1.4 with the native ''ball receptor'' in hKv1.1.

Hyperpolarization of these oocytes, on the other hand, resulted in inward currents (Fig. 3B) with kinetics similar to those observed for 4N/1, demonstrating that the chain had access to the cytoplasmic face of the transmembrane segment of the chimæra. Moreover, foreign residues in this construct are limited to the ball and chain of hKv1.4 as the T1 domain is derived entirely from hKv1.1. Since the openings in the ''hanging gondola'' (Kobertz et al. 2000) are too small to permit passage of the chain region responsible for the shift in operating range (Fig. 2C), this result clearly establishes that the ball and chain must already be present between the transmembrane channel and the co-axial T1-tetramer prior to the delivery of the depolarizing pulse. Furthermore, this location of the ball argues against the possibility of its causing inactivation by binding to the cytoplasmic mouth of the T1 pore following docking of the tetramer assembly with the channel pore. We therefore propose that the elements of the voltage-gated K^+ channel whose structures have been determined has the T1 domain hanging below the transmembrane segment and the ball and chain resident in the enclosure formed.

The N-terminus of the T1 domain is situated near the T1 pore. The C-terminus, which is linked to the transmembrane S1 segment, is on the opposite surface of the tetramer. It is generally presumed, but not proven, that this surface faces the membrane. In order for the ball to be located near the mouth of the transmembrane pore, the linking polypeptide (possibly in an extended conformation) would have to either transit the T1 pore or the window framed by the S1–T1 linkers.

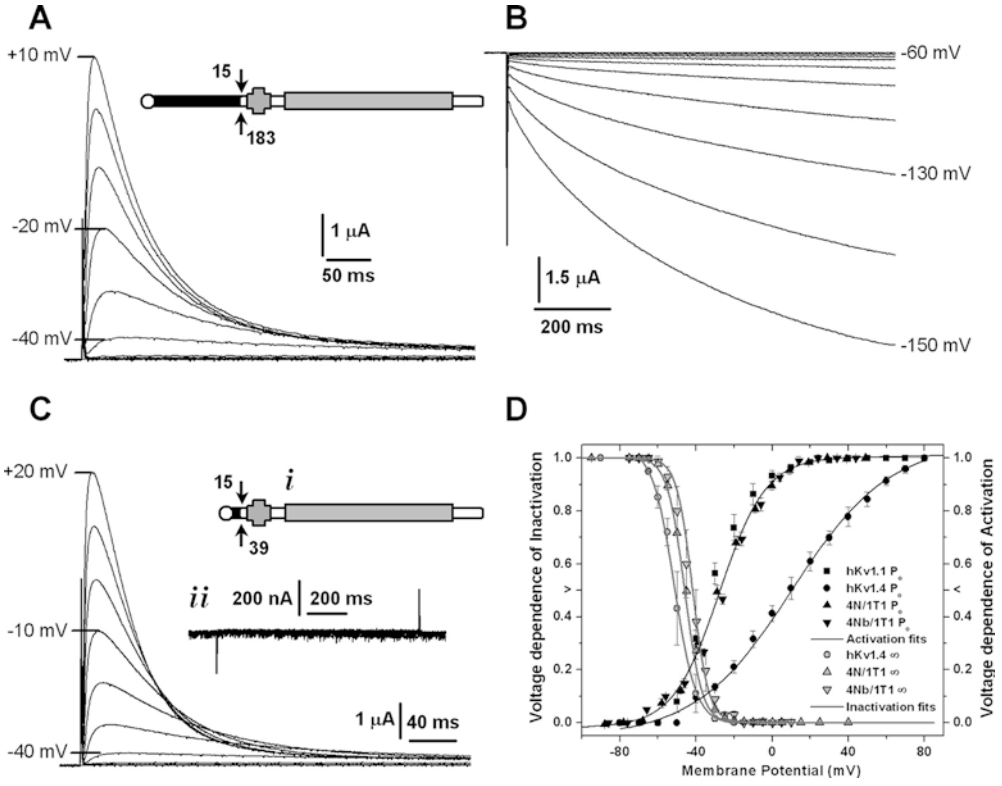

Fig. 3A–D Deletion of linker between T1 domain and inactivating ball generates outwardly rectifying channels with intact inactivation properties. **A** Rapidly inactivating outward currents through the 4N/1T1 chimæra (*inset* shows a schematic of the chimæra), in response to depolarizing pulses. No hyperpolarizing prepulse was used prior to depolarizing to potentials indicated against the traces; instead, an inter-episode time of 10 s was used to ensure complete recovery from inactivation. **B** Inward currents through 4N/1T1 channels. Oocytes expressing the 4N/1T1 chimæra were held at −65 mV and stepped to hyperpolarizing potentials from −65 to −175 mV in 10 mV steps, as shown in the inset to Fig. 2B. **C** Currents through 4Nb/1T1 chimæra (schematically shown in *inset i*), Currents elicited on depolarizing oocytes from a holding potential of −80 mV to the indicated depolarizing potentials following a 1 s hyperpolarizing prepulse of −120 mV. *Inset ii*: currents observed on hyperpolarizing to a series of potentials from −65 mV to −175 mV in −10 mV steps, from a holding potential of −65 mV. **D** Boltzmann function fits of the voltage dependence of activation (*filled symbols*) and inactivation (*open symbols*) of 4Nb/1T1 and 4N/1T1 channels compared to those of hKv1.1 and hKv1.4. The $V_{1/2}$ values used for the activation fits (*black lines*) are −29.2 mV (hKv1.1, *filled squares*), −30.5 mV (4N/1T1, *filled up triangles*) and −28.3 mV (4Nb/1T1, *filled down triangles*); $V_{1/2}$ of inactivation (*gray lines*): −42.8 mV (4Nb/1T1, *open down triangles*), −45.7 mV (4N/1T1, *open up triangles*) and −51.4 mV (hKv1.4, *open circles*)

The aberrant interactions which bring about the shift in operating range of the chimæra perturbs the structure of the channel globally as the reversal potential of the chimæra is shifted from close to −90 mV for hKv1.1 to −35 mV in the chimæra, indicative of reduced selectivity for K^+ (Chanda et al. 1999a). However, the channel is still relatively selective for K^+, as the reversal potential for *Xenopus* oocytes equally selective for Na^+ and K^+ is estimated to be about +2 mV in the ND96 bathing medium. Toxin sensitivity of the chimæra is also affected as IC_{50} for dendrotoxin (DTX) is 11 nM for the inward currents of 4N/1T1 chimæra (Varshney and Mathew 2003a) versus 3 nM for hKv1.1 (Hopkins et al. 1999). However, since DTX does not affect other Kv channels even at the micromolar concentration range (Hopkins et al. 1999), it may be concluded that the structural perturbation, while observable, is small.

In order to narrow down the portion of the transplanted hKv1.4 ball and chain responsible for the shift in operating range, we have constructed another chimæric channel, the 4Nb/1T1 chimæra, where the ball is attached to the T1 domain by a linker of only 22 residues (144 residues deleted), as opposed to 166 residues in 4N/1T1 (Fig. 3C, inset). Oocytes expressing this construct exhibit rapidly inactivating outward currents on depolarization (Fig. 3C) but no inward currents on hyperpolarization (Fig. 3C, inset). The voltage dependence of both activation and inactivation are similar to those observed for 4N/1T1 (Fig. 3D). The absence of inward currents in the 4Nb/1T1 construct suggests that the residues involved in the aberrant interaction lie either in the deleted portion of the chain, or that such a large deletion restricts the remaining residues from participation in these interactions. Identification of the membrane-embedded partners in the interaction could provide a handle to identifying the machinery underlying the transduction of voltage sensing to channel opening. More immediately germane is the rapid inactivation of the outward currents. This would suggest that the distance from the anchor site on the T1 domain to the mouth of the channel pore is comparable to or less than the length of an extended 22-residue polypeptide.

The model proposed here is at odds with those proposed elsewhere with the ball and chain suspended from the T1 domain into the cytoplasm (Aldrich 2001; Gulbis et al. 2000). Such a model is consistent with data on the inactivation of full-length Kv1.1 caused by peptides corresponding to the inactivating particles from Kv1.4, Kv3.4, ShB and Kvβ1.1 (Stephens and Robertson 1995; Antz et al. 1997; Wissmann et al. 1999). It is possible that the ''gondola window'' is large enough to permit the passage of such small peptides (ranging from 37 to 62 amino acid residues in size), but too small to permit the passage of the ball and chain from hKv1.4 (210 residues in all). Inactivating particles attached to the beta subunits by long chains are also able to snake in through the basket weave (Zhou et al. 2001). More pertinent than the length of the peptides concerned would be the structures adopted by the different peptides. The structure of 4_{BC} could be particularly bulky, given the recent NMR structure of two tandemly linked inactivating particles in the Kv1.4 channel (Wissmann et al. 2003). The size of the opening could, in principle, be estimated from sieving experiments using variants of the 4_{BC} peptide.

The model we propose is also at odds with that proposed earlier by Choe and co-workers (Kreusch et al. 1998), which has the T1 domain docking onto the cytoplasmic mouth of the channel pore and the ball binding, in turn, to the cytoplasmic mouth of the T1 pore. However, rapid N-type inactivation appears to be competitive with TEA, which binds near the selectivity filter as shown by a variety of experimental approaches (see Yellen 1998 for review). Moreover, fast inactivation by inactivation-peptides is unaffected by removal of the T1 domain of Shaker (Kobertz and Miller 1999), demonstrating that their binding site lies elsewhere.

Finally, results of chain deletion experiments on Kv1.4 indicate that the rate of inactivation is relatively insensitive to the length of the chain between T1 and the ball domain (Tseng-Crank et al. 1993). The model proposed here, with the ball residing in the restricted volume enclosed by the T1 and the transmembrane domain, would be consistent with these results. The model of Choe and co-workers would also be consistent with these data, but is ruled out by the insensitivity of inactivation to the loss of T1 (Kobertz and Miller 1999). Models with the ball and chain suspended in the cytosol require the chain to be long enough to snake around the bulky T1 assembly and present the ball to the pore. This may be inconsistent with the observed length independence of inactivation, particularly for large deletions. Tseng-Crank et al. (1993) report that deletions of up to 60 residues in the chain do not significantly reduce the rate of inactivation), while the 4Nb/1T1 construct has a chain of just 22 residues between the T1 and ball. Interestingly, Kv3.4 channels inactivate rapidly (Rudy et al. 1999), with a chain of just five residues connecting the T1 tetrameric structure (Bixby et al. 1999) and the ball structure solved by NMR (Antz et al. 1997). We estimate the length of a contour from the N-terminus of a Shaker T1 subunit radially to its outer edge and then vertically up the side of the subunit to be $\sim$50 Å (based on the crystal structure of the T1 domain). The contour length estimated from the cytoplasmic mouth of the T1 domain to the mouth of the channel pore in the Shaker EM-reconstructed structure (Sokolova et al. 2001) is $\sim$75 Å. Determining the corresponding contour on the higher resolution EM structure of the Kv1 channel is complicated by the associated β subunits, and our best estimate is $\sim$100 Å. Note that the T1 domain in this structure has a vertical dimension of almost 50 Å (Orlova et al. 2003).

Our data using physiology to assess accessibility of the cytoplasmic face of the channel to perturbing peptides clearly demonstrates that bulky peptides such as 4_{BC} are prevented from interacting with cytoplasmic residues by an assembled T1 domain. However, the same peptides when covalently attached N-terminal to the T1 domain can perturb channel physiology. We propose that the N-terminal ''ball and chain'' of voltage-gated potassium channels could be trapped within the ''hanging gondola'' formed by the suspended T1 (Kobertz et al. 2000) at the time of assembly of the channels.

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