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REVIEW

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The roles of intracellular regions in the activation of voltage-dependent potassium channels

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Abstract The involvement of the transmembrane regions S2, S3 and S4 in the activation of potassium channels by depolarization has been well clarified. However, a role of the intracellular regions in channel function is emerging. Here we review recent evidence for the roles of intracellular regions in the functioning of members of two families of channels. The Kv2.1 potassium channel, a member of the voltage activated Kv family, has long intracellular regions. By mutagenesis studies and expression in oocytes, we identify residues in both the N- and C-terminal regions that contribute to determining activation kinetics of this channel. It seems that the C-terminus wraps around the N-terminus and interacts with it functionally. The voltage-activated ether-a-go-go (eag) channels also have long intracellular regions. Despite considerable homology, eag1 and eag2 channels display different activation kinetics. By making chimeras between these channels and again expressing in oocytes, we show that residues in both the N-terminal region and the membrane-spanning region are involved in determining these differences in activation kinetics. The intracellular N- and C-terminal regions are likely to continue to prove fertile regions in future investigations into the functioning of ion channels.

Keywords Eag channels · Intracellular regions · Ion channels · Potassium channels

Introduction

The vital role that voltage-operated potassium channels (Kv) play in the functioning of cells is well known, and this ranges from control of membrane potential to modulation of action potential duration and firing frequency; they are also sites for inherited disorders (Wray 2001). Many basic elements of the structure and function of these tetrameric channels are also well understood. Each subunit comprises six membrane-spanning regions, S1 to S6, with a P region between S5 and S6; the N- and C-terminal regions are located at the intracellular side (Packer et al. 2000; Wray 2000). The channel pore is formed by the S5 and S6 regions; the selectivity filter is formed by the P region near the outer mouth of the pore and the S6 region lines the pore on the intracellular side (Fig. 1A). Upon depolarization, the positively charged S4 region is caused to move, and the negatively charged S2 and S3 regions are also involved (Yusaf et al. 1996; Cha and Bezanilla 1997; Tiwari-Woodruff et al. 1997; Milligan and Wray 2000; Bezanilla 2002; Gandhi and Isacoff 2002). The S4 regions apparently move like paddles (Jiang et al. 2003), and this causes opening of the channel gate, located at the intracellular side of the pore. The structure of the KcsA channel (Doyle et al. 1998) forms a more or less universal model for the S5–P–S6 part of the channel, and more recently a crystal structure of the whole S1 to S6 region has been published (Jiang et al. 2003).

However, the structural and functional roles of the intracellular N- and C-terminal regions are still very unclear. Here, progress on understanding the functional roles of these intracellular regions is reviewed for Kv2.1 (a voltage-operated Kv channel) and for eag channels (members of the ether-a-go-go channel family).

Kv2.1 channels

Kv channels possess a so-called T1 (“tetramerization”) domain, located within part of the N-terminal region (Fig. 1C). This region is concerned with the control of subunit–subunit assembly within families and with the binding of the auxiliary beta subunit; the T1 region is also concerned with the modulation of channel gating (Zerangue et al. 2000; Liu et al. 2001; Minor 2001;

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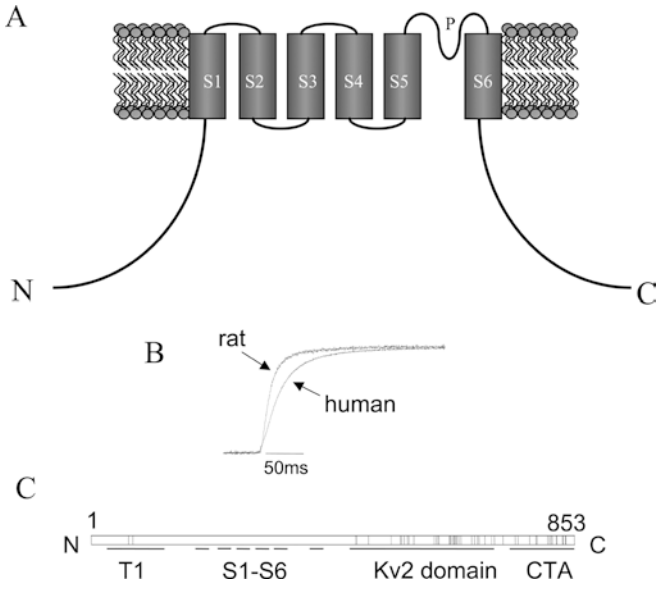

Fig. 1A–C The Kv2.1 channel. **A** Arrangement of transmembrane regions S1–S6 for voltage-dependent channels such as Kv2.1. **B** Normalized current traces for rat and human Kv2.1 channels, during voltage steps from −80 mV to +40 mV. **C** Domains of the Kv2.1 channel (853 amino acids). The positions of the T1, S1–S6 and Kv2 domains are shown by *horizontal bars*. As discussed in the text, there is also a C-terminal activation domain (CTA) occupying residues 741–853, also shown by a *horizontal bar*. Differences between the sequences for rat and human are indicated by *vertical lines*. Experimental methods throughout are as described in Ju et al. (2003)

Strang et al. 2001). For instance, mutations in this domain can shift current–voltage curves, and can affect activation and deactivation kinetics. Somehow or other, the T1 domain must also participate in gating movements. The crystal structure of this domain has been determined for several potassium channels (Kreusch et al. 1998; Bixby et al. 1999; Cushman et al. 2000; Gulbis et al. 2000; Minor et al. 2000).

Recently, electron microscopy studies with single-particle averaging for the Shaker Kv1.1 channel and a mammalian Kv1 channel have dramatically shown that the channel comprises two basic domains: a membrane-spanning domain and an intracellular domain that hangs below the membrane by four connectors, like a ''hanging gondola'' (Sokolova et al. 2001; Orlova et al. 2003). The structure that lies below the channel includes the T1 region, and, as we also suggest in our experiments reviewed here, also includes the C-terminal region. We show here that the N- and C-terminal regions interact, and both regions are concerned with channel gating processes (Ju et al. 2003).

We chose to study the Kv2.1 channel for two reasons. Firstly, this channel has longer intracellular regions than the Kv1 channel, and indeed these intracellular regions account for 73% of the number of amino acids in Kv2.1. Secondly, the rat and human forms of this channel have markedly different activation kinetics: the rat channel is fast activating but the human channel is slowly activating (Fig. 1B). However, the two channels have completely identical amino acids in the membrane-spanning region S1 to S6. Thus the differences in function between these two channels are due only to N- and C-terminal regions; we set out to locate the intracellular amino acids that are concerned with these differences in activation kinetics.

At the N-terminal region there are only two amino acids (positions 67 and 75, located in the T1 region) that are different between the rat and human forms of the Kv2.1 channel, while in the C-terminal region, 49 amino acids are different (Fig. 1C). To track down the amino acids that are involved in activation kinetics, we have made mutations and chimeras between these two channels, expressing the constructs in oocytes (Ju et al. 2003).

We first investigated the role of the two N-terminal residues:

1. Residue 67. For the rat channel, swapping the amino acid at position 67 (Q) with that for the human channel (E) altered the activation kinetics: Q gave fast activation kinetics and E slow kinetics (Fig. 2A). However, for the human channel, swapping the amino acid at this residue, in apparent contradiction, had no effect on kinetics: activation remained slow (data not shown).
2. Residue 75. For the human channel, swapping the amino acid at position 75 (D) with that in rat (E) altered the activation kinetics: D gave slow kinetics and E fast kinetics (Fig. 2A). For the rat channel, however, again in apparent contradiction, swapping amino acids at this residue did not affect the kinetics: activation remained fast (data not shown).

How are these apparently contradictory results to be explained? Clearly both residues 67 and 75 in the N-terminus are involved in altering the activation kinetics, but the differences in activation kinetics between rat and human must depend also on other residues. The only other residues that are different are those located at the C-terminus, and thus the C-terminus must also be involved, and probably interacts with the N-terminus in determining the activation kinetics.

To investigate this further, we carried out biochemical experiments to look for interactions between N- and C-terminal regions. For this, we expressed a GST fusion protein with the entire N-terminus of Kv2.1, and incubated it with the entire C-terminus protein which we had prepared by in vitro transcription and translation. On purification of the GST N-terminus fusion protein with glutathione, we found that radiolabelled C-terminus protein co-associated, indicating that an interaction between the N- and C-termini indeed occurred (Ju et al. 2003). However, further experiments using the Biacore surface plasmon resonance system to detect binding between the N- and C-termini indicated that the binding may be weak and dependent on the conditions used.

If C-terminal residues are also involved in determining activation kinetics, and if they interact with

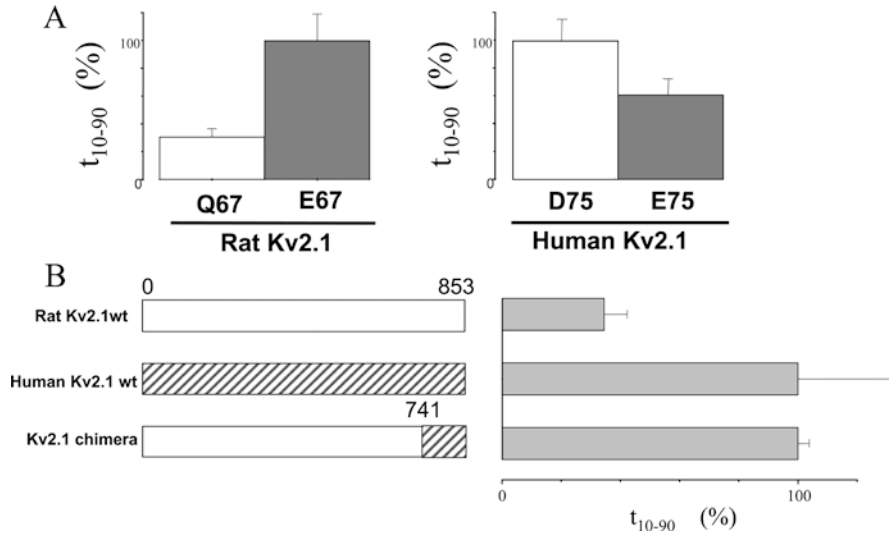

Fig. 2A, B Mutations of Kv2 channels. **A** Activation times, measured as the time for 10%–90% of maximum current, $t_{10–90}$, during voltage steps from −80 mV to 0 mV. Rise times were not significantly different between E67 rat and D75 human, and these were both normalized to 100%. Activation times are shown for the rat Kv2.1 channel with Q or E at position 67, and for the human Kv2.1 channel with D or E at position 75. **B** Activation times for rat and human wild-type channels, and for a chimera with residues 741–853 of the rat channel replaced by human sequence. Here, activation times have been normalized to human wild-type

N-terminal residues, which C-terminal residues are involved? To answer this question, we made several chimeras between rat and human Kv2.1 channels, swapping C-terminal regions (Ju et al. 2003). When we replaced amino acids 741–853 in the rat channel with the corresponding residues in the human channel, we found that the activation time was changed from fast (like rat) to slow (like human) (Fig. 2B). This suggests that amino acids within the domain 741–853 determine differences in activation times between the two channels, and we refer to this region as the C-terminal activation domain, "CTA". Further experiments (data not shown) indicated that 1–5 residues are involved in the subdomain 749–795, as well as 1–10 residues in the subdomain 796–838. Furthermore, experiments carried out with other chimeras (data not shown) indicated that other parts of the C-terminus were not involved.

By homology modelling with known structures for the T1 domain, residues 67 and 75 are apparently located at exposed positions at the outside of the T1 tetramer (Ju et al. 2003). Further modelling suggests that there may be a marked local change when residue 67 is mutated from Q to E, caused by electrostatic repulsion of the negatively charged glutamate. At residue 75, there is a change in bulk of the residue as it is mutated from D to E. It is striking that these mutations cannot affect the activation kinetics by interacting directly with the S4 region or its intracellular linkers because these residues do not in fact face the membrane. Instead, our data indicate that these residues in the T1 region interact with the CTA domain in the C-terminus. Contact between the T1 region and the C-terminus has also been hypothesized on the basis of the hydrophobicity of the outer surface of the T1 tetramer (Minor et al. 2000).

Taking all the available data into account, we have constructed a schematic model of the Kv2.1 channel (Fig. 3) (Ju et al. 2003). In this model, the "hanging gondola" comprises the T1 tetramers in the middle and is surrounded by the large C-terminal region. In particular, the CTA domain at the end of the C-terminus

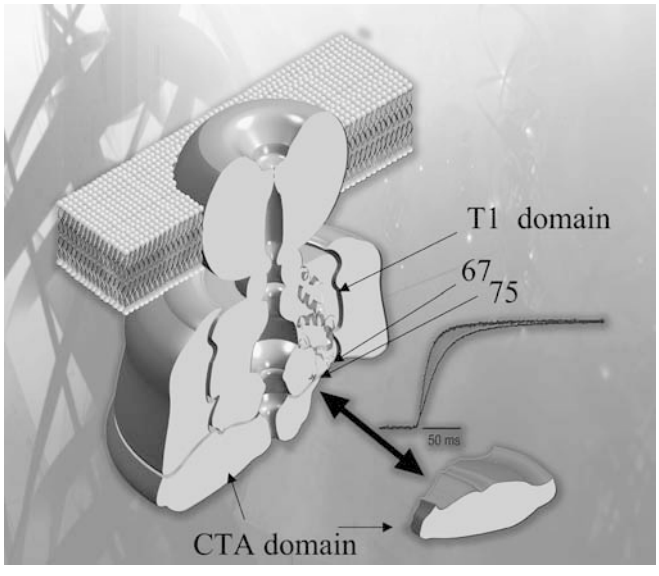

Fig. 3 Schematic cut-away representation of the Kv2.1 channel. This figure shows the membrane-spanning part of the channel, and the intracellular regions including T1 and CTA domains. Ions flowing through the channel pass through four windows between the membrane-spanning and intracellular parts (not through the pore in the intracellular part, which is shown enlarged in the diagram). The model was guided by the electron microscope structure of the Shaker channel, allowing for the larger C terminal region in Kv2.1, and was also guided by our results indicating that residues 67 and 75 at the outside face of the T1 region interact with the CTA domain in the C-terminus region (shown in *exploded view*). The predicted (Ju et al. 2003) 3D structure of the T1 domain is also shown (α helices and β strands shown)

probably overlaps with the side and lower part of the T1 domain, where residues 67 and 75 are located. The gondola itself is suspended by linkers comprising the short regions N-terminal to S1 and C-terminal to S6.

How do mutations in the T1 region affect gating? Mutations in T1, and indeed deletions, can affect gating, even when these mutations are deep within the T1 tetramer (VanDongen et al. 1990; Elkes et al. 1997; Pascual et al. 1997; Kobertz and Miller 1999; Cushman et al. 2000; Minor et al. 2000; Zerangue et al. 2000; Minor 2001) and so the effects cannot be due to direct actions on the S4 region or the pore. Indeed, previously it has been suggested (Minor et al. 2000) that the conformational change of gating involves movement of both the membrane-gating machinery and the T1 region as a whole. Our data indicate that the C-terminal region may also be involved in this conformational change: the C-terminal region may affect gating via its connection to S6 and the pore. In fact, mutations in the T1 region may entirely affect gating indirectly via the C-terminal region. However, no direct information is available at single channel level to clarify the nature of these activation conformational changes. However, one may perhaps speculate, by analogy with other perturbations of these channels (Pascual et al. 1997), that the latency of first channel opening is somehow altered by the intracellular parts of the channel. In this case, the intracellular parts may interfere with conformational changes before the first opening of the channels immediately after depolarization. Clearly these interesting possibilities will be worthy of further investigation.

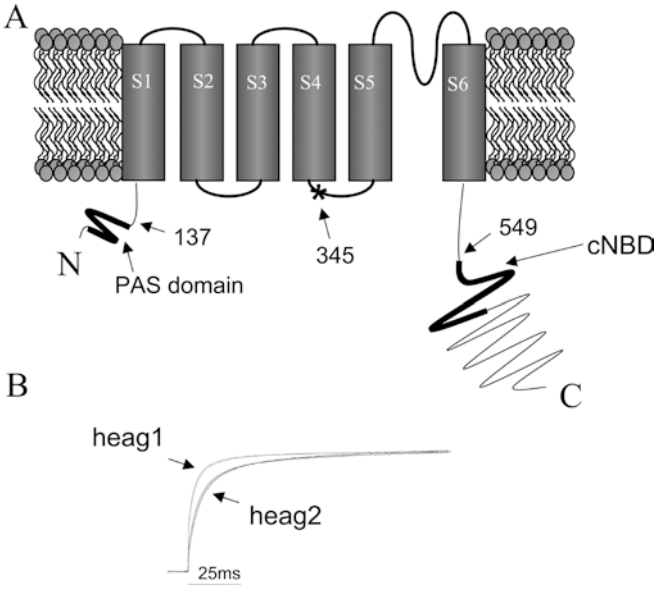

Fig. 4A–C Heag channels. **A** Arrangement of the eag channels, with S1–S6 regions in the membrane, a PAS domain in the N-terminal region and a cyclic nucleotide binding region, cNBD, in the C-terminal region. The S4 region and the S4–S5 linker are identical between heag1 and heag2 except for residue 345 indicated (I in heag1, L in heag2). Residues 137 and 549 are also indicated. **B** Sample normalized current traces showing the difference in activation times for heag1 and heag2 channels. The traces are shown for steps from holding potentials of −60 mV and −70 mV to a test potential of +40 mV; activation is slower for hyperpolarised potentials (not shown)

Eag channels

The ether-a-go-go family, named after the *Drosophila* prototype (Warmke and Ganetzky 1994), is characterized by long N- and C-terminal intracellular tails, and is subdivided into eag, elk and erg subfamilies. Within this important family, there are eight mammalian channels, eag1, eag2, elk1-3 and erg1-3 (Packer et al. 2000; Bauer and Schwarz 2001). The last member to be cloned, eag2, was obtained in rat (Saganich et al. 1999; Ludwig et al. 2000) and in human (Ju and Wray 2002; Schonherr et al. 2002).

Eag channels, eag1 and eag2, are characterized (Ludwig et al. 1994; Robertson et al. 1996; Stansfield et al. 1996; Terlau et al. 1996; Frings et al. 1998; Occhiodoro et al. 1998; Ju and Wray 2002; Schonherr et al. 2002) by outward rectification without inactivation, and by fast deactivation and slow activation. The activation kinetics depend on the holding potential (''Cole–Moore shift'') and extracellular magnesium. Structurally, eag channels comprise several distinct domains that are well conserved across the whole family (Fig. 4A). Besides the core part of the channel, which contains the six transmembrane regions and the P region, there is a ''PAS'' domain in the N-terminus and a cyclic nucleotide-binding domain, cNBD, in the C-terminus. The PAS domain is also found in many unrelated proteins, particularly transcription factors, and can mediate protein–protein interactions; it is named after three proteins where the domain was first identified: Per, Arnt and Sim (Sassoni-Corsi 1998). The crystal structure of the PAS domain in herg has been solved (Cabral et al. 1998). The cNBD was initially thought not to be of importance in channel function (Ludwig et al. 1994; Frings et al. 1998), but it now seems that, at least for herg, the cNBD binds cAMP, and may play a role in the control of herg by the auxiliary subunit Mirp1 (Cui et al. 2001).

The human eag channels (heag1 and heag2) are 73% homologous, and the membrane-spanning regions, the PAS domain and the cNBD domain are well conserved. We recently compared the functional properties of heag1 and heag2 (Ju and Wray 2002). We found that, compared with heag1, the conductance–voltage curve for heag2 was shifted to the left and the voltage sensitivity was less; also the activation kinetics were slower for heag2, and the sensitivity to blocking drugs was lower for heag2.

Here we review some of our experiments investigating the molecular basis for these differences in function between heag1 and heag2. In particular, we have studied the role of the intracellular regions on the activation kinetics of these channels (Ju and Wray 2003). For these studies, we made chimeras between heag1 and heag2 channels, swapping the N-terminal region, the central

membrane-spanning region or the C-terminal region between these channels. Constructs were expressed in oocytes and voltage-clamp recordings of channel currents made. As can be seen in Fig. 4B, activation was fast for the heag1 wild-type channel compared with heag2 wild-type. However, for chimera I, which had the first 137 amino acids of heag2 replaced by the corresponding amino acids of heag1, the resulting construct was fast-activating, like heag1 (Fig. 5). This suggests that these N-terminal residues, which include the PAS domain, play a role in determining the differences in activation kinetics between the heag channels. Furthermore, for chimera II with residues 138–549 of the heag2 channel replaced by corresponding residues from heag1, the activation kinetics were again fast, like heag1 (Fig. 5). This indicates that the central, membrane-spanning region also plays a role in determining differences in activation kinetics between the two channels. Lastly, we replaced amino acids 550–988 of the heag2 channel with the corresponding residues of the heag1 channel (chimera III). This time, the activation kinetics remained slow, suggesting that the C-terminus did not participate in determining the differences in activation kinetics between the heag1 and heag2 channels (Fig. 5).

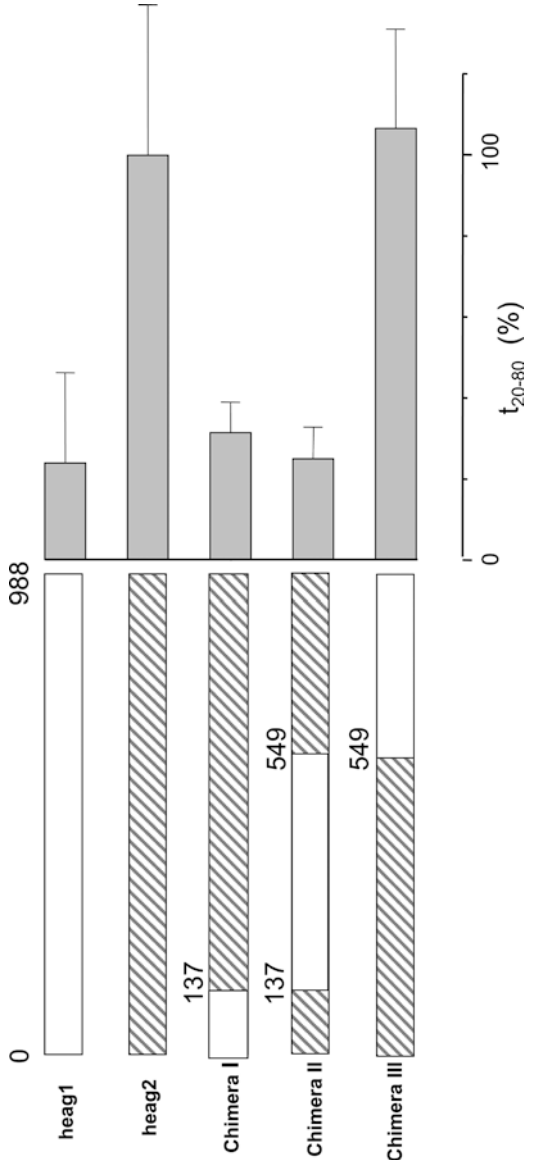

Fig. 5 Mutations of heag channels. The figure shows the activation times, measured as the time for 20%–80% of maximum current, t_{20-80}, during voltage steps from −80 mV to 0 mV. Times are shown for wild-type heag1 and heag2, as well as for chimera I with residues 1–137 of heag2 replaced by corresponding residues of heag1, for chimera II with residues 138–549 of heag2 replaced by heag1, and for chimera III with residues 550–988 of heag2 replaced by heag1. Rise times have been normalized to the value for heag2 wild-type

Thus, in summary, the data show that both the N-terminal region and the central membrane-spanning region both contribute to determining differences in the activation kinetics between heag1 and heag2 channels. Most probably this occurs by an interaction between the N-terminus and some part of the membrane-spanning region. One obvious candidate region for such an interaction is the S4–S5 linker near the mouth of the pore. Indeed, interactions between this linker and the N-terminus have been postulated to have a role in deactivation kinetics (Cabral et al. 1998; Wang et al. 1998; Sanguinetti and Xu 1999; Terlau et al. 1997). However, the amino acids of the S4–S5 linker are identical between heag1 and heag2, except for one amino acid, at residue 345 (I in heag1, L in heag2) (Fig. 4A). To investigate whether this residue plays a role in determining differences in activation between heag1 and heag2 channels, we mutated the residue, I345L in heag1, and L345I in heag2. In neither case, though, did the mutation affect the activation kinetics: the heag1 mutant remained fast (Ju et al. 2002) and the heag2 mutant remained slow. This therefore shows that this residue, and indeed the whole of the S4–S5 linker, do not participate in determining differences in activation kinetics between the two channels. Possibly the N-terminal region may interact with other parts of the channel, such as the S2–S3 loop, or the channel pore and S6.

Finally, it is worth noting that, rather than being confined to simply Kv2.1 and eag channels, intracellular regions probably play vital roles in the function of a range of channel types. Examples include KAT channels and inward rectifier channels (Marten and Hoshi 1997; Aydar and Palmer 2001; Jones et al. 2001; Nishida and MacKinnon 2002). The N- and C-terminal intracellular regions may indeed prove to be of widespread fundamental importance in channel gating machinery.

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References

Aydar E, Palmer C (2001) Functional characterization of the C-terminus of the human *ether-a-go-go*-related gene K^+ channel (HERG). J Physiol (London) 534:1–14

Bauer CK, Schwarz JR (2001) Physiology of EAG K^+ channels. J Membr Biol 182:1–15

Bezanilla F (2002) Perspective – voltage sensor movements. J Gen Physiol 120:465–473

Bixby KA, Nanao MH, Shen NV, Kreusch A, Bellamy H, Pfaffinger PJ, Choe S. (1999) Zn^{2+}-binding and molecular determinants of tetramerization in voltage-gated K^+ channels. Nat Struct Biol 6:38–43

Cabral JHM, Lee A, Cohen SL, Chait BT, Li M, Mackinnon R (1998) Crystal structure and functional analysis of the HERG potassium channel N terminus: a eukaryotic PAS domain. Cell 95:649–655

Cha A, Bezanilla F (1997) Characterizing voltage-dependent conformational changes in the *Shaker* K^+ channel with fluorescence. Neuron 19:1127–1140

Cui J, Kagan A, Qin D, Mathew J, Melman YF, McDonald TV (2001) Analysis of the cyclic nucleotide binding domain of the HERG potassium channel and interactions with KCNE2. J Biol Chem 276:17244–17251

Cushman SJ, Nanao MH, Jahng AW, DeRubeis D, Choe S, Pfaffinger PJ (2000) Voltage-dependent activation of potassium channels is coupled to T1 domain structure. Nat Struct Biol 7:403–407

Doyle DA, Morais Cabral J, Pfuetzner RA, Kuo A, Gulbis JM, Cohen SL, Chait BT, MacKinnon R (1998) The structure of the potassium channel: molecular basis of K^+ conduction and selectivity. Science 280:69–77

Elkes DA, Cardozo DL, Madison J, Kaplan JM (1997) EGL-36 *Shaw* channels regulate *C. elegans* egg-laying muscle activity. Neuron 19:165–174

Frings S, Brull N, Dzeja C, Angele A, Hagen V, Kaupp UB, Baumann A (1998) Characterization of ether-a-go-go channels present in photoreceptors reveals similarity to I_{kx}, a K^+ current in rod inner segments. J Gen Physiol 111:583–599

Gandhi CS, Isacoff EY (2002) Perspective – molecular models of voltage sensing. J Gen Physiol 120:455–463

Gulbis JM, Zhou M, Mann S, MacKinnon R (2000) Structure of the cytoplasmic beta subunit-T1 assembly of voltage-dependent K^+ channels. Science 289:123–127

Jiang Y, Lee A, Chen J, Ruta V, Cadene M, Chait BT, MacKinnon R (2003) X-ray structure of a voltage-dependent K^+ channel. Nature 423:33–41

Jones PA, Tucker SJ, Ashcroft FM (2001) Multiple sites of interaction between the intracellular domains of an inwardly rectifying potassium channel, Kir6.2. FEBS Lett 508:85–89

Ju M, Wray D (2002) Molecular identification and characterisation of the human eag2 potassium channel. FEBS Lett 524:204–210

Ju M, Wray D (2003) Activation of heag1 and heag2 channels: roles of molecular regions. Biophys J 84:545a

Ju M, Rashleigh L, Aslam M, Wray D (2002) Investigation of the role of a residue in the S4-S5 linker in the activation of heag potassium channels. J Physiol (London) 544:8P

Ju M, Stevens L, Leadbitter E, Wray D (2003) The roles of N- and C-terminal determinants in the activation of the Kv2.1 potassium channel. J Biol Chem 278:12769–12778

Kobertz WR, Miller C (1999) K^+ channels lacking the 'tetramerisation' domain: implications for pore structure. Nat Struct Biol 6:1122–1125

Kreusch A, Pfaffinger PJ, Stevens CF, Choe S (1998) Crystal structure of the tetramerisation domain of the *Shaker* potassium channel. Nature 392:945–948

Liu TI, Lebaric ZN, Rosenthal JJ, Gilly WF (2001) Neural substitutions at highly conserved T1-domain residues perturb processing and functional expression of squid Kv1 channels. J Neurophysiol 85:61–71

Ludwig J, Terlau H, Wunder F, Bruggemann A, Pardo LA, Marquardt A, Stuhmer W, Pongs O (1994) Functional expression of a rat homologue of the voltage gated ether-a-go-go potassium channel reveals differences in selectivity and activation kinetics between the Drosophila channel and its mammalian counterpart. EMBO J 13:4451–4458

Ludwig J, Weseloh R, Karschin C, Liu Q, Netzer R, Engeland B, Stansfield C, Pongs O (2000) Cloning and functional expression of rat eag2, a new member of the *ether-a-go-go* family of potassium channels and comparison of its distribution with that of eag1. Mol Cell Neurosci 16:59–70

Marten I, Hoshi T (1997) Voltage-dependent gating characteristics of the K^+ channel KAT1 depend on the N and C termini. Proc Natl Acad Sci USA 94:3448–3453

Milligan CJ, Wray, D. (2000) Local movement in the S2 region of the voltage-gated potassium channel hKv2.1 studied using cysteine mutagenesis. Biophys J 78:1852–1861

Minor DL (2001) Potassium channels: life in the post-structural world. Curr Opin Struct Biol 11:408–414

Minor DL, Lin YF, Mobley BC, Avelar A, Jan YN, Jan LY, Berger JM (2000) The polar T1 interface is linked to conformational changes that open the voltage-gated potassium channel. Cell 102:657–670

Nishida M, MacKinnon R (2002) Structural basis of inward rectification: cytoplasmic pore of the G protein-gated inward rectifier GIRK1 at 1.8 Å resolution. Cell 111:957–965

Occhiodoro T, Bernheim L, Liu J-H, Bijlenga P, Sinnreich M, Bader CR, Fischer-Lougheed J (1998) Cloning of a human *ether-a-go-go* potassium channel expressed in myoblasts at the onset of fusion. FEBS Lett 434:177–182

Orlova EV, Papakosta M, Booy FP, van Heel M, Dolly JO (2003) Voltage-gated K^+ channel from mammalian brain: 3D structure at 18 Å of the complete $(\alpha)_4(\beta)_4$ complex. J Mol Biol 326:1005–1012

Packer J, Conley E, Castle N, Wray D, January C, Patmore L (2000) Diversity of potassium channels. Trends Pharmacol Sci 21:327–326 (poster insert)

Pascual JM, Shieh CC, Kirsch GE, Brown AM (1997) Contribution of the NH_2 terminus of Kv2.1 to channel activation. Am J Physiol 273:C1849–1858

Robertson GA, Warmke JW, Ganetzky B (1996) Potassium currents expressed from *Drosophila* and mouse *eag* cDNAs in *Xenopus* oocytes. Neuropharm 35:841–850

Saganich MJ, Vega-Saenz de Miera E, Nadal MS, Baker H, Coetzee WA, Rudy B (1999) Cloning of components of a novel subthreshold-activating K^+ channel with a unique pattern of expression in the cerebral cortex. J Neurosci 19:10789–10802

Sanguinetti MC, Xu QP (1999) Mutations of the S4-S5 linker alter activation properties of HERG potassium channels expressed in *Xenopus* oocytes. J Physiol (London) 514:667–675

Sassone-Corsi P (1998) Molecular clocks: mastering time by gene regulation. Nature 392:871–874

Schonherr R, Gessner G, Lober K, Heinemann SH (2002) Functional distinction of human EAG1 and EAG2 potassium channels. FEBS Lett 514:204–208

Sokolova O, Kolmakova-Partensky L, Grigorieff N (2001) Three-dimensional structure of a voltage-gated potassium channel at 2.5 nm resolution. Structure 9:215–220

Stansfield CE, Roper J, Ludwig J, Weseloh RM, Marsh SJ, Brown DA, Pongs O (1996) Elevation of intracellular calcium by muscarinic receptor activation induces a block of voltage-activated rat ether-a-go-go channels in a stably transfected cell line. Proc Natl Acad Sci USA 93:9910–9914

Strang C, Cushman SJ, DeRubeis D, Peterson D, Pfaffinger PJ (2001) A central role for the T1 domain in voltage-gated potassium channel formation and function. J Biol Chem 276:28493–28502

Terlau H, Ludwig J, Steffan R, Pongs O, Stuhmer W, Heinemann SH (1996) Extracellular Mg^{2+} regulates activation of rat *eag* potassium channel. Pflugers Arch 432:301–312

Terlau H, Heinemann SH, Stuhmer W, Pongs O, Ludwig J (1997) Amino terminal-dependent gating of the potassium channel rat eag is compensated by a mutation in the S4 segment. J Physiol (London) 502:537–543

Tiwari-Woodruff SK, Schulteis CT, Mock AF, Papazian DM (1997) Electrostatic interactions between transmembrane segments mediate folding of *Shaker* K^+ channel subunits. Biophys J 72:1489–1500

VanDongen AM, Frech GC, Drewe JA, Joho RH, Brown AM (1990) Alteration and restoration of K^+ channel function by deletions at the N- and C-termini. Neuron 5:433–443

Wang J, Trudeau MC, Zappia AM, Robertson GA (1998) Regulation of deactivation by an amino terminal domain in *human ether-a-go-go-related gene* potassium channels. J Gen Physiol 112:637–647

Warmke JW, Ganetzky B (1994) A family of potassium channel genes related to *eag* in *Drosophila* and mammals. Proc Natl Acad Sci USA 91:3438–3442

Wray D (2000) Ion channels: molecular machines par excellence. Sci Spectra 23:64–71

Wray D (2001) Inherited disorders of ion channels: an overview. Pharm News 8(2):12–17

Yusaf SP, Wray D, Sivaprasadarao A (1996) Measurement of the movement of the S4 segment during the activation of a voltage-gated potassium channel. Pflugers Arch 433:91–97

Zerangue N, Jan YN, Jan LY (2000) An artificial tetramerisation domain restores efficient assembly of functional Shaker channels lacking T1. Proc Natl Acad Sci USA 97:3591–3595