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The Enigmatic Cytoplasmic Regions of KCNH Channels

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Abstract

KCNH channels are expressed across a vast phylogenetic and evolutionary spectrum. In humans they function in a wide range of tissues and serve as biomarkers and targets for diseases such as cancer and cardiac arrhythmias. These channels share a general architecture with other voltage-gated ion channels but are distinguished by the presence of an N-terminal Per-Arnt-Sim (PAS) domain and a C-terminal domain with homology to cyclic nucleotide binding domains (referred to as the CNBh domain). Cytosolic regions outside these domains show little conservation between KCNH families but within a family are strongly conserved across species, likely reflecting variability that confers specificity to individual channel types. PAS and CNBh domains participate in channel gating, but at least twice in evolutionary history the PAS domain has been lost, and in one family it is omitted by alternate transcription to create a distinct channel subunit. In this focused review we present current knowledge of the structure and function of these cytosolic regions, discuss their evolution as modular domains, and provide our perspective on the important questions moving forward.

Keywords

KCNH; PAS; Potassium Channels; ether-à-go-go; CNBh domains

Introduction

EAG or KCNH channels are voltage-gated potassium channels with roles in cardiac repolarization, cellular proliferation and tumor growth. In mammals, KCNH family comprises 8 channel types distributing into three subfamilies based on sequence similarity¹. EAG1 and 2 (Kv10.1-2) are orthologs of the founding *Drosophila* ether-à-go-go channel², whereas ERG (Kv11.1-3) and ELK (Kv12.1-3) represent the other two subfamilies (Fig 1A). These channels are associated with several human pathophysiological conditions. Loss of

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human (h)ERG channel function due to inherited mutations or off-target drug block is associated with long QT syndrome (LQTS) and sudden cardiac death, particularly in children and young adults³. Expression of both hERG1 and EAG1 is dramatically increased in several types of cancer, including a range of tumor and leukemic cancers, where they are considered biomarkers of proliferation and invasiveness^{4;5}. Blockers of both channels have been identified as anti-proliferative agents *in vitro* and may be useful for certain chemotherapeutic approaches. Overexpression of one hERG1 isoform has been linked to schizophrenia⁶. ELK-type channels have not yet been associated with human disease, but ELK2 knockout confers an epilepsy-like phenotype in mouse⁷. Because members of all subfamilies are extensively expressed in brain, it is reasonable to expect future studies to uncover a wide range of functionalities and pathophysiology yet to be described.

Architecture of KCNH channels

Like other voltage-gated potassium (Kv) channels, KCNH channels have six transmembrane helices comprising a voltage sensor and a potassium selectivity filter, and intracellular amino (N) and carboxyl (C) termini² (Fig. 1B). Also like other Kv channels, KCNH channels are gated by changes in membrane potential, which cause conformational changes in the voltage sensor (S1–S4 domain) that are translated to opening of the conducting pore (lower S6)^{8;9;10;11}. In particular, members of the KCNH family possess N-terminal and C-terminal cytosolic regions that are unique among Kv channels^{1;2;12}. In the N-terminus they have a Per-Arnt-Sim (PAS) domain¹³. In other proteins PAS domains are components of environmental cue sensing pathways^{14;15;16}. Although in the channel literature this domain is often, even by us, referred to as the “eag domain,” we prefer to call it the “PAS domain” to facilitate recognition of the domain by scientists outside the channel field. In the C-terminus, KCNH channels have a region of homology to cyclic nucleotide-binding (CNB) domains^{1;2;12}; this region has been called the cyclic nucleotide-binding homology (CNBh) domain^{17;18}. CNB domains are found in a wide range of proteins, including cyclic nucleotide-gated (CNG) and hyperpolarization-activated cyclic nucleotide-modulated (HCN) ion channels, where they modulate channel activity through binding of cyclic nucleotides¹⁹.

PAS domain

A surprising connection between the N-terminal region of KCNH channels and a bacterial light sensor, the photoactive yellow protein, was revealed with the determination of the three-dimensional structure of the first 135 residues of the human ERG (hERG) channel (Fig. 2A and 2B)¹³. These two proteins are now recognized as part of the large family of PAS domain proteins^{14;15;16}. PAS domains mediate protein-protein interactions and/or act as sensor proteins in pathways that modulate cellular responses to environmental cues. Many PAS domains bind a small molecule; some of these ligands are cofactors, such as p-coumaric acid (Fig. 2B) and heme (Fig. 2C). They bind tightly to the protein and undergo chemical changes that initiate signaling cascades in response to environmental cues. In other cases ligand binding is transient, e.g., the binding of C4-dicarboxylates to a PAS domain of the DctB histidine kinase, a component of the dicarboxylic acids transport system; in this instance the functional role of the PAS domain is to sense variations in ligand

concentration²⁰. There are also many PAS domains for which no small molecule ligand is known.

The PAS domain in the hERG1 (Kv11.1) channel plays a critical role in maintaining normal cardiac activity. hERG1 channels produce I_{Kr} ^{21; 22}, a potassium current important in repolarizing the ventricular action potential²³. I_{Kr} is largely inactivated at the peak of the action potential, but as repolarization is initiated under the influence of Na^+ channel inactivation and activation of other potassium currents, I_{Kr} recovers from inactivation and produces a rebounding current that slowly turns off²⁴. Slow deactivation requires the PAS domain^{25; 26}, which was shown to stabilize the mean open time of single channels²⁷. In the absence of the PAS domain, deactivation is accelerated and the resurgent current is consequently curtailed. Mutations that disrupt PAS domain structure cause functional or trafficking defects associated with long QT syndrome^{28; 29; 30}; thus, the integrity of the PAS domain is important in preventing cardiac arrhythmias and as a target of surveillance by the cell's quality control mechanisms to ensure a properly folded and assembled channel.

High resolution crystal structures of the PAS domains from the hERG¹³, mouse EAG (mEAG) and *Drosophila* ELK channels³¹ have been reported, as have several NMR structures of the hERG PAS domain^{32; 33; 34}. These structures are all very similar to each other and none has a bound small molecule. Nevertheless, hERG and mEAG PAS structures show a cavity where other PAS domains bind small molecules³⁵ (Fig. 2A). These cavities are reasonably large (~100 Å³) and display variability in their shape and volume, but lack the chemical variety and polar residues expected for a small molecule-binding site.

The first 25 amino acids of the channel (and the PAS domain) are disordered in the high-resolution crystal structure¹³ but have emerged as a unit of structural and functional importance. NMR structures of hERG channels reveal that residues 13–23 of this N-terminal “Cap” form an amphipathic α -helix while residues 1–12 remain disordered^{32; 33; 34}. The functional effect of PAS deletions on hERG deactivation was localized to the N-terminal Cap (Fig. 2D and 2E)^{13; 33; 34; 36}, and application of a peptide corresponding to the first 16 amino acids partially restored wild-type deactivation properties to channels from which the N terminus had been deleted (2–354)²⁷. In addition, in hERG a disulfide bridge can be formed between cysteine residues introduced in the N-terminal Cap and the S4–S5 linker bridging the voltage sensor domain and the channel gate³⁷ and in EAG channels, gating changes induced by a small N-terminal deletion (aa 7–12) were compensated by S4 mutations³⁸. Together, these results suggest that the N-terminal Cap interacts with the gating machinery in KCNH channels and that much of the regulation of deactivation attributed to the PAS domain likely arises from the interactions of this amino acid stretch.

CNB homology domain

The strong amino acid sequence similarity along the cytoplasmic C-terminal regions of KCNH, CNG and HCN channels was noted soon after the channel genes were first cloned and sequenced^{1; 12}. This similarity led to the reasonable proposal that KCNH channels were also regulated by cyclic nucleotides, but subsequent studies reported that cyclic nucleotides failed to directly modulate channel gating^{9; 39} or bind to the channel protein³⁹. And

although crystal structures of the CNBh domains from the zebrafish ELK (Fig. 3A)¹⁸, mouse EAG channels¹⁷ and mosquito ERG channels⁴⁰ confirmed the strong structural similarity between these domains and CNB domains (Fig. 3), clear differences explain the lack of cyclic nucleotide binding (Fig. 4A and 4B). For example, CNB domains share a highly conserved arginine that binds the cyclic phosphate and stabilizes the cyclic nucleotide in the binding pocket (Fig. 4B). In KCNH channels, the arginine is not conserved, nor is the small helix (α P helix) that in channel CNB domains mediates ligand-induced conformational changes.

This is not the only example in which CNB domains from related proteins have diverged to assume unique functions. The family of transcription factors known as CPR-FNR (cyclic AMP receptor protein-fumarate and nitrate reduction regulator) includes some members that are regulated by cyclic nucleotides and others that are not. Catabolite activator protein (CAP) is regulated by cAMP, which binds to a canonical CNB domain. However, the corresponding domains in the related transcription factors Cprk⁴¹ and CoxA⁴² have evolved to bind other small molecules - halogenated metabolites and heme, respectively. Like the CNBh domain of KCNH channels, the binding pockets of these proteins lack the critical arginine and small helix of bona fide CNB domains (Figure 4C).

Unique among all CNB and CNB-like domains are those of the KCNH channels. Instead of a cyclic nucleotide, the CNBh domain binds a C-terminal stretch of conserved amino acids that loops back to occupy the binding pocket; thus, the domain appears “self-liganded”^{17; 18}. The liganding residues uncannily mimic a cyclic nucleotide, with a tyrosine side-chain substituting for the nucleotide base and a leucine side-chain replacing the cyclic phosphate (Fig. 3, and cf. Figs. 4A and B). Mutations in this amino acid stretch alter the gating properties of KCNH channels leading to the suggestion that this is an important region for gating modulation^{17; 18; 40}. There is also some evidence that modulation of the channel can occur through competition of this intrinsic ligand by either by a small molecule, for example flavonoids⁴³, or another protein, calmodulin¹⁷, but at present it has not been clearly demonstrated that binding of either of these molecules results in the release of the intrinsic ligand sequence from its binding site.

The close sequence similarity between CNG or HCN channels and KCNH channels also extends to the C-linker, a ~60 residue region connecting the end of the last transmembrane helix (S6) to the CNBh domain (Fig. 1B). While the functional role of the C-linker in KCNH channels has yet to be thoroughly explored, it has been well demonstrated that this region is crucial for cyclic nucleotide-dependent gating of CNG and HCN channels⁴⁴. X-ray crystal structures from the mouse HCN2 channel show four C-linkers in a tetrameric “elbow-on-the shoulder” arrangement that is suggested to assemble and disassemble in a cyclic-nucleotide dependent manner^{45; 46; 47; 48}. The structures of the CNBh domains from the mosquito ERG and zebra-fish ELK channels include the totality of their C-linkers. In these structures the C-linkers retain some of the features seen in the HCN2 structure. In particular, they are largely helical and some of the contacts established between neighboring protein molecules in the crystal resemble the “elbow-on-the-shoulder” arrangement^{18; 40}. This evidence has been used to propose that in the KCNH channel the C-linkers will adopt the tetrameric assembly seen in HCN channels^{49; 50}. However, the assembly state of CNBh

domains is still unresolved as the conformation of the C-linker varies between CNBh domain structures and is different from the C-linker structure of the HCN2 channel CNB domain (cf. conformation of C-linkers in Fig. 3A and 3B); in addition, unlike CNB domains from HCN2 channels⁴⁵, the isolated CNBh domains do not form tetramers in solution or in the crystal^{18; 40}.

PAS-CNBh Complex

Early experiments demonstrated that injection of PAS domain protein into *Xenopus* oocytes expressing truncated human ERG channel results in a partial recovery of wild-type channel properties¹³. Additionally, biochemical, functional and FRET experiments showed that the isolated PAS domain assembles with the truncated channel and, more specifically, with the CNBh domain^{50; 51; 52; 53; 54}. This is an intersubunit interaction where the PAS domain from a channel subunit associates with the CNBh domain from another channel subunit⁵⁵.

A recent X-ray crystal structure of the complex between the PAS domain and the CNBh domains has finally revealed the details of this interaction⁴⁹ (Fig. 5). One of the interesting features in this structure is that the protein-protein interface involves CNBh domain residues immediately before the intrinsic ligand sequence. This suggests that the functional impact of mutations in the intrinsic ligand could result from destabilization of the PAS-CNBh complex. In this context it is worth noting that solution studies have shown that the interaction between the CNBh and PAS domains of the mouse EAG channel is weak, with a K_d around 10 μ M⁴⁹.

Another interesting aspect of the PAS-CNBh complex structure is that, apart from a loop region in the CNBh domain that is not very well ordered, there are no noticeable structural changes between the domains in the complex or as isolated structures. In particular, interaction with the PAS domain did not alter the disposition of the helices on the surface of the CNBh domain; in CNB domains ligand-dependent rearrangement of these helices is linked to activity changes in the effector protein. In addition, the structure of the complex provides a molecular explanation for several cancer-related mutations found in the human EAG1 channel and LQT2 mutations found in the PAS domain of the human ERG. The LQT2 mutants R56Q and N33T are particularly interesting because unlike other LQT2-associated mutations in the PAS domain, these mutations cause changes in the channel's functional properties^{28; 30} but do not destabilize the folding properties of the PAS domain or affect channel trafficking^{29; 30}. The PAS-CNBh structure reveals that the two residues are part of the protein-protein interface and so it is very likely that, as had been previously proposed, destabilization of the complex is the basis of the altered channel properties and the cause of the disease⁵².

KCNH Channels Lacking PAS Domains

Despite the high degree of conservation of PAS domains in KCNH channels, for some Kv11/ERG family subunits the PAS domain is specifically omitted during biosynthesis. ERG 1b is an isoform encoded by an alternate transcript of the *KCNH2* gene in which the first five exons encoding the N terminus of ERG 1a, the original isolate, are replaced with an alternate exon that encodes a unique N terminus missing the PAS domain (Fig. 6A)^{56; 57}.

hERG 1b avidly coassembles with 1a, effectively reducing the number of PAS domains in the channel assembly⁵⁸. Deactivation in the heteromer is faster than that of 1a homomers; in addition, activation and recovery from inactivation are also accelerated, resulting in currents nearly twice the magnitude during a cardiac action potential at physiological temperatures (Fig. 6B)⁵⁹. Both subunits are expressed and associate in the heart⁶⁰, and loss of the 1b subunit has been associated with long QT syndrome in an 8-year-old patient⁵⁹ and a case of intrauterine fetal death⁶¹. With this genetic mechanism, gating kinetics in hERG channels can be dialed up or down depending on the number of PAS domains present⁶². Because independent promoters regulate hERG 1a and 1b transcription, changing developmental or environmental conditions could dictate hERG subunit stoichiometry by changing gene expression. Indeed, the relative levels of 1b expression appear to be greater in the young compared with adult heart^{61; 63}.

For other ERG channels, the PAS domain has been lost in certain evolutionary lineages. The sole ERG representative in *Drosophila*, encoded by the *seizure* gene (*sei*), lacks an N-terminal PAS domain (Fig. 6A)⁶⁴. Channels produced by *sei* expression in *Xenopus* oocytes lack the I_{K_r} -like rectification of mammalian ERG channels and have fast deactivation, much like EAG channels⁶⁵. Bioinformatics and functional analysis comparing *Drosophila*, *C. elegans* and sea anemone orthologs indicate the PAS domain sequence was present in an ancestral gene predating the divergence of the bilaterians and cnidarians, but degenerated at least twice during evolution⁶⁶. Functional analyses within these lineages reveal a coordinate degeneration of sequences required for C-type inactivation, which allows the channels to gate open during depolarization like classical delayed rectifiers^{65; 66} (Fig. 6C). The CNBh domain sequence is conserved, however, reflecting selective pressures on this domain whose function independently contributes to the fitness of the organism it serves.

Other cytoplasmic regions

Outside of the amino acid regions corresponding to the PAS domain, the transmembrane domains, the C-linker and the CNBh domain, a simple analysis of the amino-acid sequence of the human ERG channel shows that there are relatively large regions predicted to have no secondary structure. These secondary structure-poor regions correspond to long amino acid stretches positioned between the PAS domain and the transmembrane domains and between the CNB homology domain and the C-terminus. The importance of the secondary structure-poor regions is not well understood. In hERG channels, truncation of the amino acid stretch between the PAS domain and transmembrane helix S1 gave rise to faster channel activation⁶⁷. This effect was largely erased when the PAS domain was also truncated, suggesting that the two regions influence each other. In rat EAG and hERG channels, a 40–50 amino acid stretch close to the C-terminus predicted to form a coiled-coil was implicated in subunit oligomerization^{68; 69}, but more recently this conclusion has been challenged⁷⁰.

Within these secondary structure-poor regions are recognition sites for signaling proteins. Arguably one of the most interesting results involving these regions is related to the mechanism of inhibition of the activity of the human EAG1 channel by Ca^{2+} /calmodulin⁷¹. This small protein binds at three sites⁷²: a high affinity site (BD-N) ~15 residues away from the C-terminal end of the PAS domain, a lower affinity site (BD-C1) immediately after the

CNBh domain, and a second high-affinity site (BD-C2) ~35 residues downstream from CNBh domain. The proximity of the calmodulin binding sites to the PAS and CNBh domain raises the possibility that the mechanism of channel inactivation by Ca^{2+} /calmodulin involves these two globular domains or affects their interaction. Consistent with this hypothesis, the BD-C1 site is partially obstructed by interactions with the PAS domain in the structure of PAS-CNBh complex ⁴⁹ and *in vitro* biochemical data indicates that binding of Ca^{2+} /calmodulin to BD-C1 induces a conformational change in the CNBh domain ¹⁷. FRET measurements with the intact channel indicate that the BD-N and BD-C2 sites, in the absence of BD-C1, are sufficient to bind Ca^{2+} /calmodulin ⁷³, but further work is needed to determine if BD-C1 plays a functional role in channel inhibition.

Perhaps related to the action of calmodulin is the interaction of the kinase CaMKII and a C-terminus site in the *Drosophila* EAG channel. EAG is specifically phosphorylated by CaMKII at a threonine residue on the C-terminal cytoplasmic region ⁷⁴. The kinase and channel form a stable complex, as demonstrated by co-immunoprecipitation from fly head extracts ⁷⁵. Interestingly, the kinase binding site in the channel strongly resembles the CAMKII binding site in the GLUN2B subunit of the ionotropic NMDA receptor channel, and both resemble the self-inhibitory sequence in CAMKII. The interaction between the NMDA receptor and CAMKII is formed in the post-synaptic density of neurons and is an essential feature of the molecular mechanism underlying long-term potentiation ⁷⁶. EAG channels are heavily expressed in the central nervous system, but modulation *in situ* by Ca^{2+} /calmodulin or CAMKII has yet to be demonstrated.

Regions lacking secondary structure may also serve as recognition sites for trafficking of the channel in the secretory pathway. A yeast two-hybrid screen revealed the cis-Golgi protein GM130 was a hERG binding partner using the carboxyl terminus as bait ⁷⁷. The interaction was disrupted by deletions of the C-terminal-most 117 amino acids. The interaction was also perturbed by LQT2 mutations V822M, S818P and R823W far upstream, but not by intervening LQT2 mutation N861I or the common LQT2-linked polymorphism K897T. Interestingly, the disrupting LQT2 mutations reside in the CNBh domain. The mutant hERG proteins are trafficking-defective, as if hERG channel exit from the ER or access to the Golgi requires the integrity of two regions, one of poor secondary structure, and the other a highly ordered domain ⁷⁷.

Unanswered questions

Despite the considerable advances brought to KCNH channel biology by structural approaches, how the cytoplasmic domains (PAS, CNBh and C-linker) from different channel subunits assemble together and communicate with the gating machinery remains unknown. Assuming that the strong sequence similarity between the C-linker regions of KCNH and HCN channels justifies proposing a similar tetrameric ring structure, it is possible to generate a C-linker/CNBh domain-PAS domain complex and to position this model on the cytoplasmic face of the structure of the Kv1.2-Kv2.1 channel chimera ⁷⁸ (Fig. 7A). A feature of this model is that the PAS domain localizes to the periphery of this gating ring, far from the voltage sensor domain or lower S6 gate of the channel. So how does the PAS domain interact with the gating machinery? NMR structures of the human ERG

channel provide a clue. We have described above (PAS domain section) the functional importance of the N-terminal Cap and how it has been deduced that this region most likely interacts with the gating machinery. In the structure of the PAS-CNBh complex⁴⁹ some of the molecules in the crystal show the N-terminal Cap interacting with the surface of the PAS domain, as shown in Fig. 7A. In the NMR structures the helix of the N-terminal Cap helix does not interact with the surface of the PAS domain but rather was modeled with multiple solutions reflecting a high degree of structural variability in this region^{32; 33; 34}. Docking one of the PAS domain NMR structures (PDB code 2LOW) onto the model shows that the N-terminal Cap is long enough to reach the voltage sensor, the S4–S5 linker or the C-linker tetrameric ring (Fig. 7B) and indicates how the PAS domain can affect the gating properties of the channel.

The model (Fig. 7B) also raises another point. In other proteins, PAS and CNB domains serve a regulatory function in which the binding of small molecule ligands or signaling proteins is transduced into conformational changes. It is not known if corresponding events in the KCNH globular domains lead to modulation of channel gating. Our structural model (Fig. 7B) raises the simpler possibility that in KCNH channels a static C-linker/CNBh-PAS complex serves only as an anchor to correctly position the N-terminal Cap. Any of the functional alterations due to mutations or truncations in the N-terminal Cap or in the entire PAS domain, or in channels naturally lacking the PAS domain, would ultimately point to a loss of N-terminal Cap function. It is interesting to note that the genetic tuning of channel properties described for several KCNH channels^{56; 57; 66} aligns with this mechanistic view of the KCNH cytoplasmic domains; functional changes result from variation of the number of PAS domains present in native channels due to alternative transcription or evolutionary sequence divergence.

Still, the structural similarities with other proteins compel us to consider the possibility of dynamic changes mediated by these cytoplasmic domains. For the CNBh domain, can the self-liganded binding pocket undergo a conformational change to become unliganded? Such a mechanism was proposed for the inhibition of EAG1 channels by Ca²⁺/calmodulin binding¹⁷ or potentiation by flavonoid binding⁴³. Similarly, KCNH PAS domains seem poised to interact with small molecules yet to be identified³⁵. In this model, modulation of the conformation of either domain would result in gating changes due to alterations in the C-linker assembly, the relative orientation of the PAS and CNBh domains or the position of the N-terminal Cap. Despite these intriguing hypotheses, direct evidence that conformational changes in either domain dynamically modulate KCNH channel gating remains to be established.

Answering these questions will require the creation of new tools and the application of additional approaches. Screening small molecule libraries may yield tools to probe or perturb specific cytoplasmic domains during channel gating. Dynamic changes may be revealed by state-dependent modification⁷⁹ or inferred from the effects of constraining domain movements with disulfide trapping⁸⁰. Optical techniques may elucidate how conformational changes in different regions of the channel contribute to concerted gating processes observed electrophysiologically. Combined with modern genetic and cell biology approaches, these approaches may ultimately reveal how the cytoplasmic domains enable

KCNH channels to fulfill their physiological roles in their native, and diverse, cellular environments.

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References

1. Warmke JW, Ganetzky B. A family of potassium channel genes related to eag in *Drosophila* and mammals. *Proc Natl Acad Sci U S A*. 1994; 91:3438–42. [PubMed: 8159766]
2. Warmke J, Drysdale R, Ganetzky B. A distinct potassium channel polypeptide encoded by the *Drosophila* eag locus. *Science*. 1991; 252:1560–2. [PubMed: 1840699]
3. Sanguinetti MC, Tristani-Firouzi M. hERG potassium channels and cardiac arrhythmia. *Nature*. 2006; 440:463–9. [PubMed: 16554806]
4. Crociani O, Guasti L, Balzi M, Becchetti A, Wanke E, Olivotto M, Wymore RS, Arcangeli A. Cell cycle-dependent expression of HERG1 and HERG1B isoforms in tumor cells. *J Biol Chem*. 2003; 278:2947–55. [PubMed: 12431979]
5. Pardo LA, Stuhmer W. The roles of K(+) channels in cancer. *Nat Rev Cancer*. 2014; 14:39–48. [PubMed: 24336491]
6. Huffaker SJ, Chen J, Nicodemus KK, Sambataro F, Yang F, Mattay V, Lipska BK, Hyde TM, Song J, Rujescu D, Giegling I, Mayilyan K, Proust MJ, Soghoyan A, Caforio G, Callicott JH, Bertolino A, Meyer-Lindenberg A, Chang J, Ji Y, Egan MF, Goldberg TE, Kleinman JE, Lu B, Weinberger DR. A primate-specific, brain isoform of KCNH2 affects cortical physiology, cognition, neuronal repolarization and risk of schizophrenia. *Nat Med*. 2009; 15:509–18. [PubMed: 19412172]
7. Zhang X, Bertaso F, Yoo JW, Baumgartel K, Clancy SM, Lee V, Cienfuegos C, Wilmot C, Avis J, Hunyh T, Daguia C, Schmedt C, Noebels J, Jegla T. Deletion of the potassium channel Kv12.2 causes hippocampal hyperexcitability and epilepsy. *Nat Neurosci*. 2010; 13:1056–8. [PubMed: 20676103]
8. Ludwig J, Terlau H, Wunder F, Bruggemann A, Pardo LA, Marquardt A, Stuhmer W, Pongs O. Functional expression of a rat homologue of the voltage gated either a go-go potassium channel reveals differences in selectivity and activation kinetics between the *Drosophila* channel and its mammalian counterpart. *EMBO J*. 1994; 13:4451–8. [PubMed: 7925287]
9. Robertson GA, Warmke JM, Ganetzky B. Potassium currents expressed from *Drosophila* and mouse eag cDNAs in *Xenopus* oocytes. *Neuropharmacology*. 1996; 35:841–50. [PubMed: 8938715]
10. Wynia-Smith SL, Gillian-Daniel AL, Satyshur KA, Robertson GA. hERG gating microdomains defined by S6 mutagenesis and molecular modeling. *J Gen Physiol*. 2008; 132:507–20. [PubMed: 18955593]
11. Thouta S, Sokolov S, Abe Y, Clark SJ, Cheng YM, Claydon TW. Proline scan of the HERG channel S6 helix reveals the location of the intracellular pore gate. *Biophys J*. 2014; 106:1057–69. [PubMed: 24606930]
12. Guy HR, Durell SR, Warmke J, Drysdale R, Ganetzky B. Similarities in amino acid sequences of *Drosophila* eag and cyclic nucleotide-gated channels. *Science*. 1991; 254:730. [PubMed: 1658932]
13. Morais Cabral JH, Lee A, Cohen SL, Chait BT, Li M, Mackinnon R. Crystal structure and functional analysis of the HERG potassium channel N terminus: a eukaryotic PAS domain. *Cell*. 1998; 95:649–55. [PubMed: 9845367]
14. McIntosh BE, Hogenesch JB, Bradfield CA. Mammalian Per-Arnt-Sim proteins in environmental adaptation. *Annu Rev Physiol*. 2010; 72:625–45. [PubMed: 20148691]
15. Moglich A, Ayers RA, Moffat K. Structure and signaling mechanism of Per-ARNT-Sim domains. *Structure*. 2009; 17:1282–94. [PubMed: 19836329]
16. Henry JT, Crosson S. Ligand-binding PAS domains in a genomic, cellular, and structural context. *Annu Rev Microbiol*. 2011; 65:261–86. [PubMed: 21663441]

17. Marques-Carvalho MJ, Sahoo N, Muskett FW, Vieira-Pires RS, Gabant G, Cadene M, Schonherr R, Morais-Cabral JH. Structural, biochemical, and functional characterization of the cyclic nucleotide binding homology domain from the mouse EAG1 potassium channel. *J Mol Biol.* 2012; 423:34–46. [PubMed: 22732247]
18. Brelidze TI, Carlson AE, Sankaran B, Zagotta WN. Structure of the carboxy-terminal region of a KCNH channel. *Nature.* 2012; 481:530–3. [PubMed: 22230959]
19. Rehmann H, Wittinghofer A, Bos JL. Capturing cyclic nucleotides in action: snapshots from crystallographic studies. *Nat Rev Mol Cell Biol.* 2007; 8:63–73. [PubMed: 17183361]
20. Nan B, Liu X, Zhou Y, Liu J, Zhang L, Wen J, Zhang X, Su XD, Wang YP. From signal perception to signal transduction: ligand-induced dimeric switch of DctB sensory domain in solution. *Mol Microbiol.* 2010; 75:1484–94. [PubMed: 20149110]
21. Sanguinetti MC, Jiang C, Curran ME, Keating MT. A mechanistic link between an inherited and an acquired cardiac arrhythmia: HERG encodes the IKr potassium channel. *Cell.* 1995; 81:299–307. [PubMed: 7736582]
22. Trudeau MC, Warmke JW, Ganetzky B, Robertson GA. HERG, a human inward rectifier in the voltage-gated potassium channel family. *Science.* 1995; 269:92–5. [PubMed: 7604285]
23. Sanguinetti MC, Jurkiewicz NK. Delayed rectifier outward K⁺ current is composed of two currents in guinea pig atrial cells. *Am J Physiol.* 1991; 260:H393–9. [PubMed: 1899980]
24. Zhou Z, Gong Q, Ye B, Fan Z, Makielski JC, Robertson GA, January CT. Properties of HERG channels stably expressed in HEK 293 cells studied at physiological temperature. *Biophys J.* 1998; 74:230–41. [PubMed: 9449325]
25. Schonherr R, Heinemann SH. Molecular determinants for activation and inactivation of HERG, a human inward rectifier potassium channel. *J Physiol.* 1996; 493 (Pt 3):635–42. [PubMed: 8799887]
26. Spector PS, Curran ME, Zou A, Keating MT, Sanguinetti MC. Fast inactivation causes rectification of the IKr channel. *J Gen Physiol.* 1996; 107:611–9. [PubMed: 8740374]
27. Wang J, Myers CD, Robertson GA. Dynamic control of deactivation gating by a soluble amino-terminal domain in HERG K(+) channels. *J Gen Physiol.* 2000; 115:749–58. [PubMed: 10828248]
28. Chen J, Zou A, Splawski I, Keating MT, Sanguinetti MC. Long QT syndrome-associated mutations in the Per-Arnt-Sim (PAS) domain of HERG potassium channels accelerate channel deactivation. *J Biol Chem.* 1999; 274:10113–8. [PubMed: 10187793]
29. Harley CA, Jesus CS, Carvalho R, Brito RM, Morais-Cabral JH. Changes in channel trafficking and protein stability caused by LQT2 mutations in the PAS domain of the HERG channel. *PLoS One.* 2012; 7:e32654. [PubMed: 22396785]
30. Gianulis EC, Trudeau MC. Rescue of aberrant gating by a genetically encoded PAS (Per-Arnt-Sim) domain in several long QT syndrome mutant human ether-a-go-go-related gene potassium channels. *J Biol Chem.* 2011; 286:22160–9. [PubMed: 21536673]
31. Adaixo R, Morais-Cabral JH. Crystallization and preliminary crystallographic characterization of the PAS domains of EAG and ELK potassium channels. *Acta Crystallogr Sect F Struct Biol Cryst Commun.* 2010; 66:1056–9.
32. Li Q, Gayen S, Chen AS, Huang Q, Raida M, Kang C. NMR solution structure of the N-terminal domain of hERG and its interaction with the S4–S5 linker. *Biochem Biophys Res Commun.* 2010; 403:126–32. [PubMed: 21055387]
33. Ng CA, Hunter MJ, Perry MD, Mobli M, Ke Y, Kuchel PW, King GF, Stock D, Vandenberg JJ. The N-terminal tail of hERG contains an amphipathic alpha-helix that regulates channel deactivation. *PLoS One.* 2011; 6:e16191. [PubMed: 21249148]
34. Muskett FW, Thouta S, Thomson SJ, Bowen A, Stansfeld PJ, Mitcheson JS. Mechanistic insight into human ether-a-go-go-related gene (hERG) K⁺ channel deactivation gating from the solution structure of the EAG domain. *J Biol Chem.* 2011; 286:6184–91. [PubMed: 21135103]
35. Adaixo R, Harley CA, Castro-Rodrigues AF, Morais-Cabral JH. Structural properties of PAS domains from the KCNH potassium channels. *PLoS One.* 2013; 8:e59265. [PubMed: 23555008]
36. Wang J, Trudeau MC, Zappia AM, Robertson GA. Regulation of deactivation by an amino terminal domain in human ether-a-go-go-related gene potassium channels. *J Gen Physiol.* 1998; 112:637–47. [PubMed: 9806971]

37. de la Pena P, Alonso-Ron C, Machin A, Fernandez-Trillo J, Carretero L, Dominguez P, Barros F. Demonstration of physical proximity between the N terminus and the S4-S5 linker of the human ether-a-go-go-related gene (hERG) potassium channel. *J Biol Chem.* 2011; 286:19065–75. [PubMed: 21474444]
38. Terlau H, Heinemann SH, Stuhmer W, Pongs O, Ludwig J. Amino terminal-dependent gating of the potassium channel rat eag is compensated by a mutation in the S4 segment. *J Physiol.* 1997; 502 (Pt 3):537–43. [PubMed: 9279806]
39. Brelidze TI, Carlson AE, Zagotta WN. Absence of direct cyclic nucleotide modulation of mEAG1 and hERG1 channels revealed with fluorescence and electrophysiological methods. *J Biol Chem.* 2009; 284:27989–97. [PubMed: 19671703]
40. Brelidze TI, Gianulis EC, DiMaio F, Trudeau MC, Zagotta WN. Structure of the C-terminal region of an ERG channel and functional implications. *Proc Natl Acad Sci U S A.* 2013; 110:11648–53. [PubMed: 23801759]
41. Levy C, Pike K, Heyes DJ, Joyce MG, Gabor K, Smidt H, van der Oost J, Leys D. Molecular basis of halorespiration control by CprK, a CRP-FNR type transcriptional regulator. *Mol Microbiol.* 2008; 70:151–67. [PubMed: 18717788]
42. Lanzilotta WN, Schuller DJ, Thorsteinsson MV, Kerby RL, Roberts GP, Poulos TL. Structure of the CO sensing transcription activator CooA. *Nat Struct Biol.* 2000; 7:876–80. [PubMed: 11017196]
43. Carlson AE, Brelidze TI, Zagotta WN. Flavonoid regulation of EAG1 channels. *J Gen Physiol.* 2013; 141:347–58. [PubMed: 23440277]
44. Craven KB, Zagotta WN. CNG and HCN channels: two peas, one pod. *Annu Rev Physiol.* 2006; 68:375–401. [PubMed: 16460277]
45. Zagotta WN, Olivier NB, Black KD, Young EC, Olson R, Goux E. Structural basis for modulation and agonist specificity of HCN pacemaker channels. *Nature.* 2003; 425:200–5. [PubMed: 12968185]
46. Craven KB, Zagotta WN. Salt bridges and gating in the COOH-terminal region of HCN2 and CNGA1 channels. *J Gen Physiol.* 2004; 124:663–77. [PubMed: 15572346]
47. Johnson JP Jr, Zagotta WN. The carboxyl-terminal region of cyclic nucleotide-modulated channels is a gating ring, not a permeation path. *Proc Natl Acad Sci U S A.* 2005; 102:2742–7. [PubMed: 15710893]
48. Hua L, Gordon SE. Functional interactions between A' helices in the Clinker of open CNG channels. *J Gen Physiol.* 2005; 125:335–44. [PubMed: 15738051]
49. Haitin Y, Carlson AE, Zagotta WN. The structural mechanism of KCNH-channel regulation by the eag domain. *Nature.* 2013; 501:444–8. [PubMed: 23975098]
50. Gustina AS, Trudeau MC. HERG potassium channel regulation by the N-terminal eag domain. *Cell Signal.* 2012; 24:1592–8. [PubMed: 22522181]
51. Stevens L, Ju M, Wray D. Roles of surface residues of intracellular domains of heag potassium channels. *Eur Biophys J.* 2009; 38:523–32. [PubMed: 19172261]
52. Gustina AS, Trudeau MC. A recombinant N-terminal domain fully restores deactivation gating in N-truncated and long QT syndrome mutant hERG potassium channels. *Proc Natl Acad Sci U S A.* 2009; 106:13082–7. [PubMed: 19651618]
53. Gustina AS, Trudeau MC. The eag domain regulates hERG channel inactivation gating via a direct interaction. *J Gen Physiol.* 2013; 141:229–41. [PubMed: 23319729]
54. Gustina AS, Trudeau MC. hERG potassium channel gating is mediated by N- and C-terminal region interactions. *J Gen Physiol.* 2011; 137:315–25. [PubMed: 21357734]
55. Gianulis EC, Liu Q, Trudeau MC. Direct interaction of eag domains and cyclic nucleotide-binding homology domains regulate deactivation gating in hERG channels. *J Gen Physiol.* 2013; 142:351–66. [PubMed: 24043860]
56. London B, Trudeau MC, Newton KP, Beyer AK, Copeland NG, Gilbert DJ, Jenkins NA, Satler CA, Robertson GA. Two isoforms of the mouse ether-a-go-go-related gene coassemble to form channels with properties similar to the rapidly activating component of the cardiac delayed rectifier K⁺ current. *Circ Res.* 1997; 81:870–8. [PubMed: 9351462]

57. Lees-Miller JP, Kondo C, Wang L, Duff HJ. Electrophysiological characterization of an alternatively processed ERG K⁺ channel in mouse and human hearts. *Circ Res.* 1997; 81:719–26. [PubMed: 9351446]
58. Phartiyal P, Jones EM, Robertson GA. Heteromeric assembly of human ether-a-go-go-related gene (hERG) 1a/1b channels occurs cotranslationally via N-terminal interactions. *J Biol Chem.* 2007; 282:9874–82. [PubMed: 17272276]
59. Sale H, Wang J, O'Hara TJ, Tester DJ, Phartiyal P, He JQ, Rudy Y, Ackerman MJ, Robertson GA. Physiological properties of hERG 1a/1b heteromeric currents and a hERG 1b-specific mutation associated with Long-QT syndrome. *Circ Res.* 2008; 103:e81–95. [PubMed: 18776039]
60. Jones EM, Roti Roti EC, Wang J, Delfosse SA, Robertson GA. Cardiac IKr channels minimally comprise hERG 1a and 1b subunits. *J Biol Chem.* 2004; 279:44690–4. [PubMed: 15304481]
61. Crotti L, Tester DJ, White WM, Bartos DC, Insolia R, Besana A, Kunic JD, Will ML, Velasco EJ, Bair JJ, Ghidoni A, Cetin I, Van Dyke DL, Wick MJ, Brost B, Delisle BP, Facchinetti F, George AL, Schwartz PJ, Ackerman MJ. Long QT syndrome-associated mutations in intrauterine fetal death. *JAMA.* 2013; 309:1473–82. [PubMed: 23571586]
62. Larsen AP, Olesen SP, Grunnet M, Jespersen T. Characterization of hERG1a and hERG1b potassium channels—a possible role for hERG1b in the I (Kr) current. *Pflugers Arch.* 2008; 456:1137–48. [PubMed: 18504605]
63. Wang X, Xu R, Abernathey G, Taylor J, Alzghoul MB, Hannon K, Hockerman GH, Pond AL. Kv11.1 channel subunit composition includes MinK and varies developmentally in mouse cardiac muscle. *Dev Dyn.* 2008; 237:2430–7. [PubMed: 18729211]
64. Titus SA, Warmke JW, Ganetzky B. The *Drosophila* erg K⁺ channel polypeptide is encoded by the seizure locus. *J Neurosci.* 1997; 17:875–81. [PubMed: 8994042]
65. Fergestad T, Sale H, Bostwick B, Schaffer A, Ho L, Robertson GA, Ganetzky B. A *Drosophila* behavioral mutant, down and out (dao), is defective in an essential regulator of Erg potassium channels. *Proc Natl Acad Sci U S A.* 2010; 107:5617–21. [PubMed: 20212103]
66. Martinson AS, van Rossum DB, Diatta FH, Layden MJ, Rhodes SA, Martindale MQ, Jegla T. Functional evolution of Erg potassium channel gating reveals an ancient origin for IKr. *Proc Natl Acad Sci U S A.* 2014; 111:5712–7. [PubMed: 24706772]
67. Viloria CG, Barros F, Giraldez T, Gomez-Varela D, de la Pena P. Differential effects of amino-terminal distal and proximal domains in the regulation of human erg K(+) channel gating. *Biophys J.* 2000; 79:231–46. [PubMed: 10866950]
68. Ludwig J, Owen D, Pongs O. Carboxy-terminal domain mediates assembly of the voltage-gated rat ether-a-go-go potassium channel. *EMBO J.* 1997; 16:6337–45. [PubMed: 9400421]
69. Jenke M, Sanchez A, Monje F, Stuhmer W, Weseloh RM, Pardo LA. C-terminal domains implicated in the functional surface expression of potassium channels. *EMBO J.* 2003; 22:395–403. [PubMed: 12554641]
70. Chen IH, Hu JH, Jow GM, Chuang CC, Lee TT, Liu DC, Jeng CJ. Distal end of carboxyl terminus is not essential for the assembly of rat Eag1 potassium channels. *J Biol Chem.* 2011; 286:27183–96. [PubMed: 21646358]
71. Schonherr R, Lober K, Heinemann SH. Inhibition of human ether a go-go potassium channels by Ca(2+)/calmodulin. *EMBO J.* 2000; 19:3263–71. [PubMed: 10880439]
72. Ziechner U, Schonherr R, Born AK, Gavrilova-Ruch O, Glaser RW, Malesevic M, Kullertz G, Heinemann SH. Inhibition of human ether a go-go potassium channels by Ca2+/calmodulin binding to the cytosolic N- and C-termini. *FEBS J.* 2006; 273:1074–86. [PubMed: 16478480]
73. Goncalves JT, Stuhmer W. Calmodulin interaction with hEAG1 visualized by FRET microscopy. *PLoS One.* 2010; 5:e10873. [PubMed: 20523736]
74. Wang Z, Wilson GF, Griffith LC. Calcium/calmodulin-dependent protein kinase II phosphorylates and regulates the *Drosophila* eag potassium channel. *J Biol Chem.* 2002; 277:24022–9. [PubMed: 11980904]
75. Sun XX, Hodge JJ, Zhou Y, Nguyen M, Griffith LC. The eag potassium channel binds and locally activates calcium/calmodulin-dependent protein kinase II. *J Biol Chem.* 2004; 279:10206–14. [PubMed: 14699099]

76. Sanhueza M, Lisman J. The CaMKII/NMDAR complex as a molecular memory. *Mol Brain*. 2013; 6:10. [PubMed: 23410178]
77. Roti EC, Myers CD, Ayers RA, Boatman DE, Delfosse SA, Chan EK, Ackerman MJ, January CT, Robertson GA. Interaction with GM130 during HERG ion channel trafficking. Disruption by type 2 congenital long QT syndrome mutations. *Human Ether-a-go-go-Related Gene. J Biol Chem*. 2002; 277:47779–85. [PubMed: 12270925]
78. Long SB, Tao X, Campbell EB, MacKinnon R. Atomic structure of a voltage-dependent K⁺ channel in a lipid membrane-like environment. *Nature*. 2007; 450:376–82. [PubMed: 18004376]
79. Akabas MH, Stauffer DA, Xu M, Karlin A. Acetylcholine receptor channel structure probed in cysteine-substitution mutants. *Science*. 1992; 258:307–10. [PubMed: 1384130]
80. Bass RB, Butler SL, Chervitz SA, Gloor SL, Falke JJ. Use of site-directed cysteine and disulfide chemistry to probe protein structure and dynamics: applications to soluble and transmembrane receptors of bacterial chemotaxis. *Methods Enzymol*. 2007; 423:25–51. [PubMed: 17609126]
81. Herzberg IM, Trudeau MC, Robertson GA. Transfer of rapid inactivation and sensitivity to the class III antiarrhythmic drug E-4031 from HERG to M-eag channels. *J Physiol*. 1998; 511 (Pt 1): 3–14. [PubMed: 9679158]

Research Highlights

- Aberrant KCNH function is associated with cardiac arrhythmia, epilepsy and cancer
- Structural and functional studies show PAS and CNB homology domains interacting
- Disruption of PAS or CNB homology domain alters channel gating
- PAS domains are absent in some lineages and some KCNH subunit isoforms
- Functional roles of KCNH cytoplasmic domains remain elusive

Figure 1.

A) Scheme showing the relationship among KCNH family members including the IUPHAR nomenclature for each subfamily. Drosophila members are shown in magenta; all others are representatives found in vertebrate species. B) Cartoon representation of KCNH channel architecture. Different channel regions and domains are labeled. Transmembrane helices labeled S1 to S6.

Figure 2.

Characteristics of PAS domains. PAS domain structures from A) human ERG channel (PDB code: 4HQA); B) Photoactive yellow protein (PYP) (PDB code: 2PHY), with ligand p-coumaric acid; C) FixL(1XJ2), with ligand heme. Binding pocket in PAS domain of human ERG channel is shown as grey wireframe. Truncation of PAS domain result in functional alterations in channels, D) Scaled tail currents from $\Delta 2-16$ and WT channels show that the small deletion increases deactivation rate. E) Fast deactivation time constants for WT ($n > 10$), $\Delta 2-354$ ($n > 10$), and $\Delta 2-12$ ($n = 6$) channels indicate that the two deletions have similar effects on deactivation kinetics. Adapted from ³⁶.

Figure 3. CNBh and CNB domains shown in similar orientations. A) Cyclic-nucleotide binding homology domain from zebra fish ELK channel (PDB code: 3UKN). B) cyclic-nucleotide binding domain from HCN2 channel (1Q3E). C-linker regions are shown in tan, β -rolls are shown in blue, α C helices are shown in magenta and ligands (intrinsic ligand and cAMP) are shown as yellow stick and indicated with arrows. α P and α C helices are labeled.

Figure 4.

Binding pockets of CNB-like and CNB domains. A) CNBh domain from KCNH channel (PDB code: 3UKN), with intrinsic ligand as yellow stick; B) CNB domain from HCN2 channel (1Q3E) with cAMP; C) Cprk protein (3E6C) with the ligand (3-chloro-4-hydroxyphenyl) acetic acid. Side-chains of some of the amino acids forming the binding pocket are shown as light-grey stick. Conserved arginine in CNB domains is indicated by arrow. αP helix of CNB domains is labeled.

Figure 5.
PAS-CNBh complex from mouse EAG channel (PDB code 4LLO). PAS domain on the left (brick-red and cyan), CNBh domain on the right (dark blue with intrinsic ligand red and C-terminus green).

Figure 6.

Genetic tuning of channel properties. A) Schematic showing PAS, hydrophobic core, and cyclic nucleotide binding homology domain (ψ). Top, general architecture for hERG 1a and most family members; middle, hERG 1b is identical to hERG 1a except for the N terminus, which has unique sequence (red) and lacks a PAS domain; bottom, *Drosophila* ERG (seizure) lacks a PAS domain. B) Heteromeric channels with fewer PAS domains produce larger currents due to faster activation and recovery from inactivation. Currents were evoked using a voltage clamp command generated from a ventricular action potential, and normalized to the respective peak cellular conductance measured following a step to +60 mV. From ⁵⁹. C) Top, In hERG, rapid inactivation suppresses current at depolarizing voltages in hERG. Upon repolarization, channels recover from inactivation and current rebounds before slowly deactivating. Bottom, In *Drosophila* ERG (sei), loss of PAS domain is accompanied by loss of rapid inactivation owing to coordinate amino acid changes in the domain regulating C-type inactivation. Adapted from ^{65; 81}.

Figure 7.

Model of KCNH channel. A) Different channel regions are colored and labeled. Only 1 PAS domain (out of 4) is shown. The N-terminal Cap helix (12 residues long) is shown as green CPK spheres and is positioned against the body of the PAS domain (magenta). The first 11 residues of the N-terminal Cap are not observed in the domain crystal structure (PDB code 4LLO) used to create the model. Putative limits of lipid bilayer are shown by horizontal lines. B) Channel regions labeled and colored as in left panel. PAS domain shown is one of the NMR models from PDB code 2LOW. The whole N-terminal Cap sequence (26 residues long) is shown as green CPK spheres and in an extended position, away from the body of the domain, conveying the idea that it can reach many of the gating regions of the channel. Arrow indicates beginning of the N-terminal Cap helix.