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COMMENTARY

N- and C-terminal interactions in KCNH channels: The spotlight on the intrinsic ligand

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Human ether-à-go-go related gene (hERG) channels are widely studied potassium channels because of their importance for repolarization of the cardiac action potential (Sanguinetti and Tristani-Firouzi, 2006). Inhibition of hERG channel currents due to inherited mutations or by drugs can lead to long-QT syndrome, a cardiac arrhythmia that may cause loss of consciousness and sudden cardiac death (Curran et al., 1995). hERG channels belong to the KCNH family of potassium-selective, voltage-gated ion channels that also includes ether-à-go-go (EAG) and EAG-like (ELK) channels (Warmke and Ganetzky, 1994). KCNH channels are assembled from four subunits, each of which contains a voltage sensor (VS) domain formed from the S1–S4 transmembrane segments and a central pore formed by S5–S6 (Warmke and Ganetzky, 1994; Wang and MacKinnon, 2017). KCNH channels also contain two structured intracellular domains per subunit: the N-terminal Per-Arnt-Sym (PAS) domain and the C-terminal cyclic nucleotide-binding homology (CNBH) domain. The PAS and CNBH domains interact to confer key functional features, such as slow deactivation in hERG channels (Gustina and Trudeau, 2011; Gianulis et al., 2013). Importantly, the interaction interface between the PAS and CNBH domains harbors many cardiac arrhythmia–associated mutations in hERG channels (Haitin et al., 2013). A recent study by Codding and Trudeau in the *Journal of General Physiology* reveals that the intrinsic ligand, a conserved stretch of amino acids in the CNBH domain, is required for the PAS and CNBH domain interaction in hERG channels (Codding and Trudeau, 2018).

The intrinsic ligand was discovered in the first crystal structures of the isolated CNBH domains of EAG and ELK channels (Brelidze et al., 2012; Marques-Carvalho et al., 2012). These structures revealed that the cavity formed by antiparallel β-strands, where cyclic nucleotides would typically bind in other cyclic nucleotide-binding (CNB) domains, such as CNB domains of hyperpolarization-activated cyclic nucleotide-modulated (HCN) channels, is occupied by a novel β-strand in KCNH channels. The novel β-strand and a few adjacent residues are conserved in KCNH channels and function as an intrinsic ligand, with two key residues of the β-strand structurally mimicking cAMP bound to HCN channels. These two residues are Y740 and L742 in zebrafish ELK (zELK) channels, Y699 and L701 in human EAG (hEAG) channels, and F860 and L862 in hERG channels. The aromatic residues are positioned where the purine ring of cAMP binds to HCN channels and the leucines where the cyclic phosphate of cAMP binds. Mutations of the intrinsic ligand affect gating (opening and closing) of KCNH channels, just like an extrinsic ligand would do (Brelidze et al., 2012, 2013; Marques-Carvalho et al., 2012; Zhao et al., 2017). The presence of the intrinsic ligand explains the lack of cyclic nucleotide modulation in KCNH channels. Unlike HCN and cyclic nucleotide-gated (CNG) channels, which are regulated by direct binding of cyclic nucleotides to their CNB domains, KCNH channels are not directly regulated by cyclic nucleotides due to the steric hindrance of binding by the intrinsic ligand (Robertson et al., 1996; Brelidze et al., 2009). The intrinsic ligand also interferes with the effects of flavonoids, which need to displace the intrinsic ligand in order to potentiate EAG channel currents (Carlson et al., 2013).

In their new study, Codding and Trudeau (2018) uncover a novel role of the intrinsic ligand as a structural glue that brings together the PAS and CNBH domains in KCNH channels. The investigators took advantage of the previously established FRET-based assay that reports on structural interactions between the PAS and CNBH domains (Gustina and Trudeau, 2009) and an electrophysiology-based in vitro assay that reports on functional interactions between these domains (Gustina and Trudeau, 2013). The FRET-based assay involves coexpression of an isolated PAS domain fused to cyan fluorescent protein (CFP) and hERG channels with the deletion of the PAS domain (hERGΔPAS) with the distal C terminus fused to Citrine (Gustina and Trudeau, 2009, 2013). Any structural interaction between the PAS domain tagged with the CFP donor and the CNBH domain coupled to the Citrine acceptor leads to FRET. The

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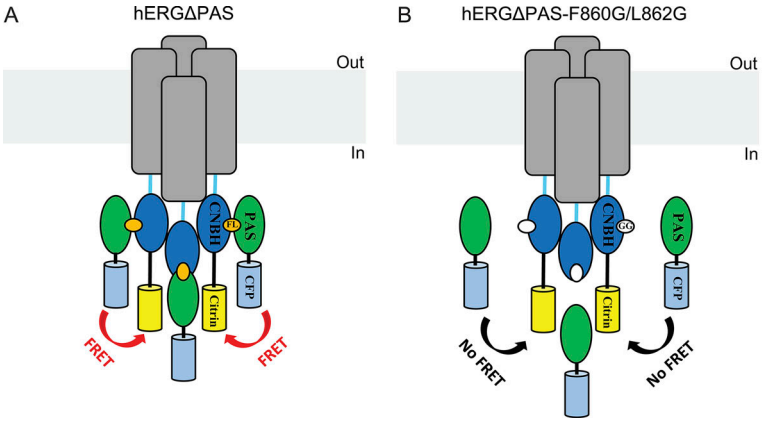

Figure 1. **Schematic of the FRET-based assay used by Codding and Trudeau (2018). (A)** FRET signal is detected between hERG channels with the deletion of the PAS domain (hERGΔPAS) tagged with Citrine at the C terminus and isolated PAS domains tagged with CFP. **(B)** No FRET signal is detected between hERGΔPAS channels with the GG mutations in the intrinsic ligand and isolated PAS domains. The PAS domain is green, CNBD blue, the C-linker light blue, CFP cyan, Citrine yellow, and transmembrane portions of the channel are colored in gray. The intact intrinsic ligand is orange and GG mutant is white.

electrophysiology-based assay is based on the observation that hERGΔPAS channels deactivate approximately five times faster than wild-type hERG channels (Morais Cabral et al., 1998). Coexpression of the isolated PAS domain and hERGΔPAS channels fully rescues the slow deactivation present in the wild-type channels with the intact PAS domain due to the functional interaction between the isolated PAS domains and CNBH domains of the hERGΔPAS channels (Gustina and Trudeau, 2013). Armed with these structural and functional indicators of PAS and CNBH domain interactions, Codding and Trudeau (2018) investigated the contribution of the intrinsic ligand.

Mutations of key residues in the intrinsic ligand affected both structural and functional interactions between the PAS and CNBH domains of hERG channels. Whereas robust FRET was detected between the CFP-tagged isolated PAS domain and hERGΔPAS channels with the Citrine-tagged C terminus (Fig. 1 A), the FRET signal was substantially reduced for hERGΔPAS channels with the F860A mutation and was virtually zero for hERGΔPAS channels with the L862A mutation or F860G/L862G double mutation (Figs. 1 B and 2 in Codding and Trudeau [2018]). The decrease in FRET efficiency indicates that the F860A mutation in the intrinsic ligand hinders structural interaction between the PAS and CNBH domains, while the F862A and F860G/L862G mutations completely prevent interactions between these domains. The functional readout for the mutations in the two residues echoed the FRET-based results. Whereas hERGΔPAS channels coexpressed with the isolated PAS domains had deactivation time constants similar to the wild-type channels, coexpression of hERGΔPAS channels with the F860A mutation and the isolated PAS domains only partially rescued the slow deactivation seen in wild-type channels. Moreover, coexpression of hERGΔPAS channels with the L862A mutation or F860G/L862G double mutations with the isolated PAS domains produced deactivation time constants that were statistically indistinguishable from those of hERGΔPAS channels (Fig. 3 in Codding and Trudeau [2018]). Mutations in the intrinsic ligand specifically affected channel deactivation kinetics, without any statistically significant effects on voltage-dependent gating or activation kinetics. Collectively, the FRET-based and electrophysiology-based results presented by Codding and Trudeau (2018) indicate that the intrinsic ligand is necessary for establishing structural and functional contacts between the PAS and CNBH domains of hERG channels.

How does this new role of the intrinsic ligand fit in the global scheme of hERG channel regulation by the intracellular PAS and CNBH domains? Recent structures of "full-length" hERG and rat EAG (rEAG) channels containing the transmembrane segments and most of the N- and C-terminal regions provide several important clues. First, the full-length structures confirmed that the PAS and CNBH domains from adjacent subunits interact in KCNH channels, and that the intrinsic ligand (Fig. 2 A, orange) occupies the β-roll cavity and is at the interface between the PAS and CNBH domains (Fig. 2 A, green and blue; Haitin et al., 2013; Whicher and MacKinnon, 2016; Wang and MacKinnon, 2017; James and Zagotta, 2018). Second, the full-length structures revealed that the S4–S5 linker is so short in KCNH channels that the VSs interact with the pore domains of the same subunits,

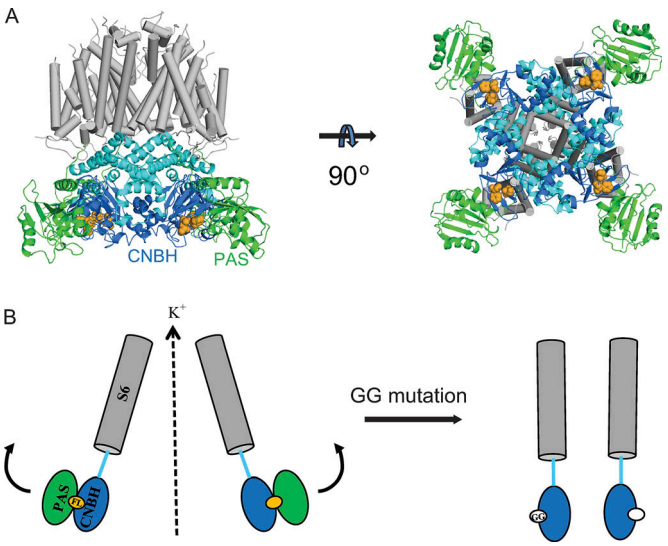

Figure 2. **Intrinsic ligand as a key element in hERG channel gating. (A)** Ribbon representation of the full-length structure of hERG channels (PDB accession no. 5VA2) viewed from the side and down the pore of the channel. The intrinsic ligand is shown in a space-filling model. The figures were prepared using PyMol (Schrodinger, LLC). **(B)** Hypothesized pulling/rotation motion regulated by the intrinsic ligand. Only two opposing subunits are shown for clarity. The color coding scheme in A and B is the same as in Fig. 1.

whereas in other unrelated potassium channels of known structure, the interaction is typically between the VSs and pore domains of adjacent subunits. As proposed by Mackinnon's group, this allows the intracellular domains to affect the pore domain independently of the VSs and could explain why mutations in the intrinsic ligand in hERG channels do not affect the voltage for half-maximal activation ($V_{1/2}$; Brelidze et al., 2013; Codding and Trudeau, 2018). Third, the full-length structures showed that the PAS domains are located at the periphery and close to the VSs, whereas the CNBH domains form an inner tetrameric ring located at the entrance to the intracellular cavity and are directly connected to the pore of the channel via the C-linkers (Fig. 2 A, light blue). By mediating structural interactions between the PAS and CNBH domains, as revealed in the study by Codding and Trudeau (2018), the intrinsic ligand drives these two domains from adjacent subunits closer. One could speculate that this will, in turn, apply a pulling or rotating force on the S6, which could translate into widening of the helical bundle at the intracellular entrance to the pore and a stabilization of the open state of the channel (Fig. 2 B, left). If the intrinsic ligand is mutated, as in the study by Codding and Trudeau (2018), the PAS and CNBD domains fail to interact and apply the pulling/rotating force to the S6, speeding up the deactivation and stabilizing the closed state of the channel (Fig. 2 B, right). Comparison of the full-length structures of rEAG in a presumably closed conformation and hERG in a presumably open conformation lends further support to this hypothesis as it suggests a rotation of the C-linker/CNBD domains during channel gating (Whicher and MacKinnon, 2016; Wang and MacKinnon, 2017; James and Zagotta, 2018). A structure of hERG channels with the pore domain in a closed state may provide valuable insight into the intrinsic ligand-dependent structural rearrangements associated with channel gating.

Mutations of the conserved Y and L amino acids in the β strand also stabilize the closed state in hEAG channels and zELK channels when currents are recorded in the inside-out configuration (Brelidze et al., 2012; Marques-Carvalho et al., 2012; Zhao et al., 2017). However, the effects of the mutations are multilayered, and there are notable differences for KCNH subfamilies. Unlike in ERG channels, the mutations in EAG channels do not affect the deactivation rate, and instead slow down activation. Also, mutations in the intrinsic ligand of EAG and ELK shift the $V_{1/2}$ to more depolarized potentials and affect the voltage-dependent steps in channel gating (Brelidze et al., 2012; Zhao et al., 2017). The molecular mechanisms of these differences are unclear, and a multidisciplinary approach based on the combination of structural, fluorescence, electrophysiology, and MD methods will be required to identify the precise steps and pathways involved in intrinsic ligand-dependent gating in KCNH channels. To spice things up even more, a recent study in zELK channels revealed that the intrinsic ligand is not a static element but undergoes dynamic structural changes that lead to its dual function as an agonist or an antagonist, depending on the voltage-dependent state of the channel and current recording configuration (Dai et al., 2018). It remains to be seen if the intrinsic ligand itself undergoes conformational changes with channel gating in other KCNH channels.

In summary, Codding and Trudeau (2018) used an elegant and conceptually simple approach to show how a seemingly minor, but strategically placed, structural motif can play a major role in domain interactions and gating regulation in hERG channels. Their study provides a crucial piece of information for formulating an integrated picture of hERG channel gating.

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