Unlocking the mechanisms of HCN channel gating with locked-open and locked-closed channels

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Ion channels are highly specialized to respond to a wide range of environmental stimuli, including transmembrane voltage and chemical ligands (Hille, 2001). The response of channels to external cues causes a change in their ion conduction, which, in turn, modifies the behavior of excitable and nonexcitable cells. The hyperpolarization-activated and cyclic nucleotide–modified (HCN) family of ion channels, which are important for spontaneous, pacemaking behavior in the heart and neurons, are regulated by both transmembrane voltage and direct binding of cyclic nucleotides (e.g., cAMP) ligands (Brown and DiFrancesco, 1980; Mayer and Westbrook, 1983; DiFrancesco and Tortora, 1991).

HCN channels are composed of four subunits, each containing six transmembrane domains (6TM). HCN channels are similar in primary structure to other 6TM channels (Gauss et al., 1998; Ludwig et al., 1998; Santoro et al., 1998), including voltage-activated potassium (Kv) channels and cyclic nucleotide–gated (CNG) channels (Kaupp et al., 1989; Warmke and Ganetzky, 1994). Similar to CNG channels, HCN contains a C-terminal cyclic nucleotide–binding domain (Zagotta et al., 2003). The mechanisms underlying the regulation of HCN channels and other 6TM channels are the subject of intensive work. Some common mechanistic themes are shared by 6TM channels: they have a pore region that is selective for particular ions, a gate domain that opens to allow the flow of ions or closes to restrict the flow of ions, and sensory domains that interact with stimuli. The mechanism by which sensory domains are coupled to gates is not well understood. Two studies by the Yellen laboratory in the September 2012 issue (Kwan et al., 2012) and in this issue (see Ryu and Yellen) of the *JGP* cast new light on the structural mechanism and energetics of coupling of the voltage sensor to the gate in HCN channels.

These new findings are particularly notable in that gating in HCN channels differs markedly from that of closely related 6TM channels. A hallmark of the 6TM domain Kv and Ca^{2+}-activated K (BK) channels is that they are all activated (opened) by depolarizing voltages and closed by hyperpolarizing voltages. In contrast, HCN channels are activated (opened) by hyperpolarizing voltages and closed by depolarizing voltages.

The voltage sensor of HCN and K channels undergoes similar movements

What is the mechanism by which HCN channels exhibit the "opposite" voltage dependence of Kv and BK channels? Initially, one potential explanation for this difference was the nature of the voltage-sensor domain itself. But, like Kv channels, the voltage-sensor domain of HCN is likely composed of the S1–S4 transmembrane domains and contains a series of positively charged residues at approximately every third position in the fourth transmembrane domain (S4) (Männikkö et al., 2002). Furthermore, HCN voltage-sensor movement is similar to that of Kv channels. S4 movement in HCN (Männikkö et al., 2002) and Kv channels (Larsson et al., 1996) was monitored by making site-directed cysteine mutations at various positions in the S4 domain and measuring the reactivity of cysteine residues to methanethiosulfonate (MTS) reagents at depolarized and hyperpolarized voltages. The accessibility of cysteine residues to MTS reagents was similar for HCN and Kv channels, suggesting that voltage sensors move outward with depolarization and inward with hyperpolarization in both HCN and Kv channels. If not the voltage sensor, then what is responsible for the opposite voltage sensitivity of HCN channels? The answer most likely lies in differences in the way that the voltage sensor is coupled to the opening and closing (gating) machinery of the HCN channel.

Molecular determinants of the gate

Voltage-sensor movement in Kv channels appears to be coupled to pore opening through the S4–S5 linker region and the lower part of the S6 region beneath the K channel pore (Lu et al., 2002; Tristani-Firouzi et al., 2002; Pathak et al., 2007) (Fig. 1, top). Structural information based on Kv1.2 channels also shows proximity of the S4–S5 linker with the S6 region of the same subunit (Long et al., 2005). In HCN channels, the S4–S5 linker,

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S6, and C-linker (a region linking the S6 domain to the cyclic nucleotide–binding domain) regions have also been implicated in voltage-dependent gating (Chen et al., 2001; Rothberg et al., 2003; Decher et al., 2004; Bell et al., 2009) (Fig. 1, bottom). The structure that gates the flow of ions also appears to be in the same place (the bottom of the S6 domain) in HCN channels and Kv channels (Liu et al., 1997; Doyle et al., 1998; Rothberg et al., 2002). Thus, not only are the voltage sensor and voltage-sensor movement similar in HCN and Kv channels, the molecular regions involved in voltage-dependent gating are also similar. Thus, the prevailing hypothesis for the opposite voltage sensitivity of HCN channels and Kv channels is that the voltage sensor must be coupled to the gate differently. But, what exactly about the coupling is different?

The structural mechanism of gating differs between HCN and Kv channels

New insights into the mechanism of coupling comes from "lock-open" and "lock-closed" HCN channels, in which experimental conditions favor either the open or closed state of the channel. To perform lock-open and lock-closed experiments, the Yellen group introduced pairs of cysteine mutations at specific sites and recorded currents in the absence and then the presence

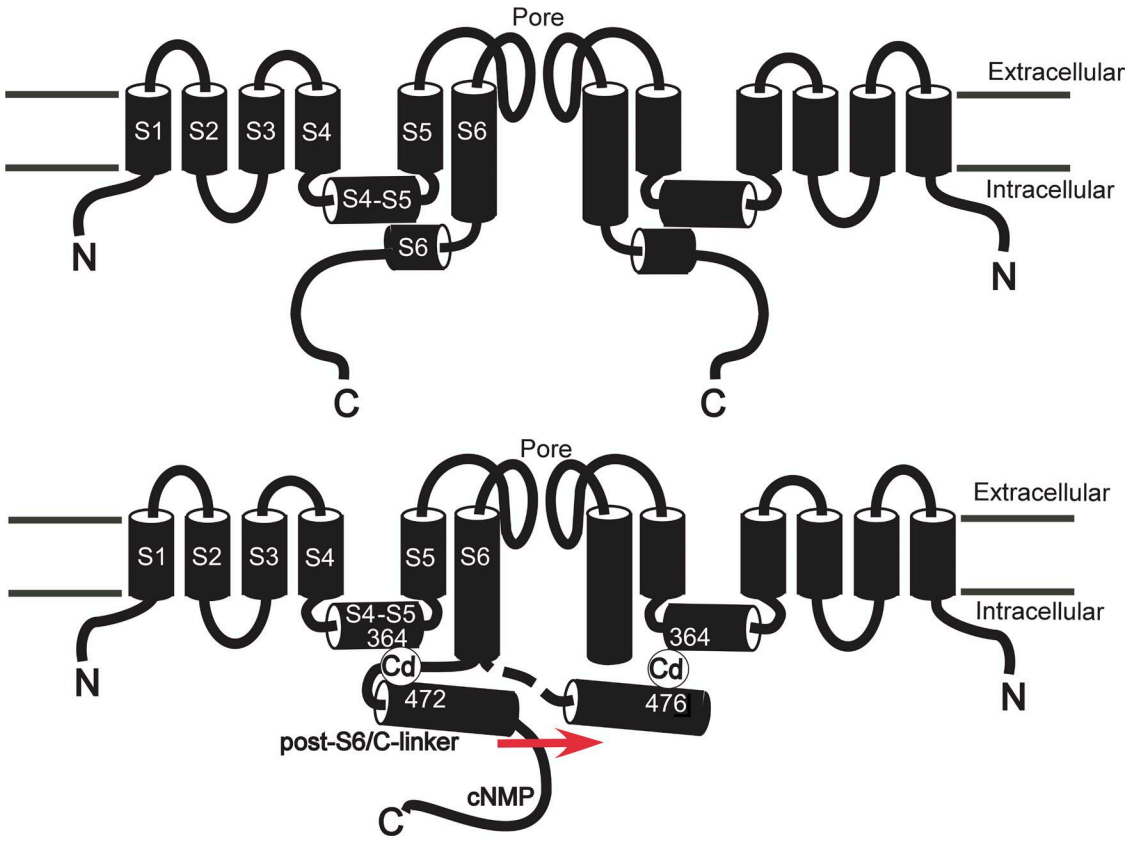

Figure 1. Proposed interdomain interactions that make up the activation gate in Kv channels and HCN channels. (Top) Schematic showing two subunits of a four-subunit Kv channel. The S1–S4 transmembrane domains make up the voltage-sensing domain, which is linked to the pore-forming regions (S5–S6 domains) via the S4–S5 linker region. Potassium ions are conducted through the central pore region. Amino and carboxyl termini are intracellular. The lower part of the S6 domain is depicted as a separate cylinder. The lower part of the S6 domain is positioned beneath the S4–S5 linker from the same subunit. In response to hyperpolarization, the S4 of the voltage sensor moves downward and pushes down on the S4–S5 linker region, which, in turn, pushes down on the lower part of the S6 domain, which brings the S6 domains closer together to narrow and close the channel gate, which restricts ion flow. (Bottom) Schematic showing two subunits of a four-subunit HCN channel. Like Kv channels, HCN channels have an S1–S4 voltage-sensor domain linked via an S4–S5 linker to the pore-forming S5 and S6 domains. Like Kv channels, ions are conducted through a central pore. N- and C-terminal regions are intracellular. Distinct from Kv channels, the C-terminal region of HCN channels contains a post-S6/C-linker domain and a cyclic nucleotide–binding domain. Cyclic nucleotide monophosphate (cNMP) is depicted as bound to the channel. (The C-terminal region of the HCN subunit on the right is cut away for clarity.) Intrasubunit interactions are depicted between 364 in the S4–S5 linker region and 472 of the post-S6/C-linker region. This interaction takes place in the open state. The dashed line connected to the post-S6/C-linker domain indicates that amino acid 476 of the same post-S6/C-linker domain makes an intersubunit interaction with 364 on an adjacent subunit. This interaction takes place in the open state. Cd^{2+} bridges indicate the close proximity of the S4–S5 linker and post-S6/C-linker regions. The red arrow indicates movement from a lock-open (364 linked to 472) to a lock-closed (364 linked to 476) state.

of nanomolar levels of Cd^{2+} ion. A functional change in channel gating suggests that the Cd^{2+} ion forms a metal bridge between two cysteine residues (Holmgren et al., 1998; Shin et al., 2004; Prole and Yellen, 2006), and implies that the two cysteine residues come into close proximity.

Kwan et al. (2012) used this technique to examine the proximity between three sites in the S4–S5 linker and eight sites in the post-S6 and adjacent A′ helix of the C-linker domain of the sea urchin HCN channel, spHCN. The authors introduced one cysteine mutation in the S4–S5 linker and a second cysteine mutation at a site in the S6/C-linker region and added Cd^{2+} to induce metal bridge formation (Kwan et al., 2012). Extending their earlier findings (Prole and Yellen, 2006), they found that multiple S4–S5 linker sites were in close proximity to S6/C-linker sites. They also found that the S6/C-linker sites that had lock-open and locked-closed effects were interleaved; for instance, residue 364C (located in the S4–S5 linker) had a lock-open effect with 472C, a locked-closed effect with 474C, and a lock-open effect with residue 482C. To explain these results, the authors proposed the provocative idea that one part of the S4–S5 linker contacts the S6/C-linker in the same subunit, and another part of the S4–S5 linker contacts the S6/C-linker of an adjacent subunit (Fig. 1, bottom). Testing this proposal with concatenated dimers, they found some sites that were consistent with intrasubunit interactions and some sites that were consistent with intersubunit interactions (Fig. 1). They interpreted the pattern of intrasubunit and intersubunit interactions to mean that movements of the S4–S5 linkers relative to the S6 region in HCN channels were different from those proposed for Kv1.2 channels, where the S4–S5 linker of a subunit sits above the lower S6 region of the same subunit, likely making only an intrasubunit interaction (Fig. 1, top) (Long et al., 2005; Pathak et al., 2007). This implies that the precise structural interactions among the very similar domains involved in HCN and Kv channel gating may produce very different responses to voltage.

The functional data from the Cd^{2+} bridge experiments in HCN channels do not fit well with existing structural and structural modeling results from Kv channels. Structural and computational data suggest that to close Kv channels, the voltage sensor pushes down on the S4–S5 linker region and that the S4–S5 linker pushes down (i.e., inward toward the cytoplasm) on the lower part of the S6 domain, thereby restricting the opening of the channel gate and reducing the flow of ions (Fig. 1, top). In contrast, for HCN channels the S4–S5 linker (in particular site 364C) makes locked-open intrasubunit interactions (with 472C), locked-closed intersubunit interactions (with 476), and locked-open intersubunit interactions (with 482C and/or 485C) in the lower S6 (Fig. 1, bottom). To explain these results, the authors propose a structural model of gating in which, with hyperpolarization, the voltage sensor moves downward and moves the S4–S5 linker, especially the lower S5 portion. Movement of the lower S5 allows S6 helices to rotate and move outward from the central axis of the channel, causing channel opening. This fundamental new mechanism for HCN channel gating raises many testable questions. Ideally, new structural information about the S4–S5 linker and S6 gate region of HCN channels would help to test the structural models derived from functional data.

The energetics of coupling differs between HCN and Kv channels

The energetics of coupling between the voltage sensor and the gate in HCN channels are also not well understood. Voltage-sensor movement in Kv channels is strict; in other words, voltage-sensor movement is tightly coupled to channel opening. For instance, in Kv channels, it is thought that the activation (or "up" configuration) of all four voltage sensors is necessary for the channel gate to open (Zagotta et al., 1994; Gagnon and Bezanilla, 2009). Therefore, at very negative voltages, when the voltage sensors are "down" or resting, the probability of Shaker K channels opening is low (Islas and Sigworth, 1999). Strict coupling of the voltage-sensor movement to the gate implies that when the gate is held open (as with an inactivation particle; Bezanilla et al., 1991), gating charge movement (and thus voltage-sensor movement) is immobilized. Linear gating models, that is to say systems in which there are a sequential number of closed states before an open state, are often sufficient to describe gating behavior in strictly coupled channels (Zagotta et al., 1994).

The Yellen group previously discovered that the link between the voltage sensor and the activation gate might be weak. They found that, in response to a hyperpolarizing voltage command (in the absence of cAMP), spHCN channels first activated and then reclosed as a result of an uncoupling or slippage between the activated voltage sensor and the gate (Shin et al., 2004). In other words, despite the HCN voltage sensor being in a "down" conformation (the activated conformation for HCN channels) because of hyperpolarization, the channel gate slipped closed and the channels did not conduct ionic currents. Other laboratories have also tested the voltage dependence of HCN channel gating and have had success with fitting a Monod–Wyman–Changeux (MWC) model to the voltage-dependent activation kinetics similar to one used to investigate voltage-activated gating in BK channels (Cox et al., 1997). In the MWC models for HCN channels (Altomare et al., 2001; Wang et al., 2002; Bruening-Wright et al., 2007), the channels can undergo an opening transition (i.e., go from the closed to the open state) that is stabilized by a constant value for each voltage sensor that goes from a resting to an active state.

Likewise, channels in an open state stabilize the transition of voltage sensors from a resting state to an active state. In MWC models, unlike the linear models for Shaker K channels where voltage-sensor movement is obligatory for channel opening, channels can also go from the closed to open state in the absence of voltage-sensor movement. Using fluorophores to label the S4 domain of HCN channels, Bruening-Wright et al. (2007) determined that movement of two voltage sensors was sufficient to activate HCN channels, an observation inconsistent with sequential models of activation but consistent with MWC models of activation.

In another recent study, Ryu and Yellen (2012) determined the coupling factor between HCN voltage sensors and activation gates by measuring gating currents (currents associated with the movement of the voltage sensor) from lock-open or lock-closed HCN channels. This approach allowed them to isolate measurements from channels with deactivated voltage sensors or channels with activated voltage sensors. Isolating the state of the voltage sensors allowed them to simplify the 10-state MWC model and more directly determine a coupling constant (a measure of the ability of the voltage sensor to affect channel opening and the ability of opening to affect the state of the voltage sensor). They found that gating charge moved more easily (was activated at less negative voltages) in the locked-open channels and less easily (was activated at more negative voltages) in locked-closed channels.

The coupling factor of the voltage sensor to the gate was much smaller in HCN channels (7.2 ± 3.0–fold per voltage sensor in lock-open channels) than in Kv channels (>100-fold per voltage sensor) (Islas and Sigworth, 1999) or BK channels (15-fold per voltage sensor). The voltage-sensor coupling to the activation gate in HCN channels is weaker than in Kv channels and may be more like that of ligand-binding coupling to the activation gate of ligand-gated channels.

The significance of the much weaker coupling in HCN compared with voltage-gated K channels likely means that other factors, such as cyclic nucleotides, can make an energetic contribution to open the HCN channel gate. This is a fascinating idea, and it will be interesting to determine whether, similar to models of voltage and Ca^{2+} activation of BK channels (Horrigan and Aldrich, 2002), cAMP activation of HCN can be added as a module to the mathematical models of voltage-dependent HCN channel gating.

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REFERENCES

Altomare, C., A. Bucchi, E. Camatini, M. Baruscotti, C. Viscomi, A. Moroni, and D. DiFrancesco. 2001. Integrated allosteric model of voltage gating of HCN channels. *J. Gen. Physiol.* 117:519–532. http://dx.doi.org/10.1085/jgp.117.6.519

Bell, D.C., H.K. Turbendian, M.T. Valley, L. Zhou, J.H. Riley, S.A. Siegelbaum, and G.R. Tibbs. 2009. Probing S4 and S5 segment proximity in mammalian hyperpolarization-activated HCN channels by disulfide bridging and Cd2+ coordination. *Pflugers Arch.* 458:259–272. http://dx.doi.org/10.1007/s00424-008-0613-3

Bezanilla, F., E. Perozo, D.M. Papazian, and E. Stefani. 1991. Molecular basis of gating charge immobilization in Shaker potassium channels. *Science.* 254:679–683. http://dx.doi.org/10.1126/science.1948047

Brown, H., and D. DiFrancesco. 1980. Voltage-clamp investigations of membrane currents underlying pace-maker activity in rabbit sino-atrial node. *J. Physiol.* 308:331–351.

Bruening-Wright, A., F. Elinder, and H.P. Larsson. 2007. Kinetic relationship between the voltage sensor and the activation gate in spHCN channels. *J. Gen. Physiol.* 130:71–81. http://dx.doi.org/10.1085/jgp.200709769

Chen, J., J.S. Mitcheson, M. Tristani-Firouzi, M. Lin, and M.C. Sanguinetti. 2001. The S4-S5 linker couples voltage sensing and activation of pacemaker channels. *Proc. Natl. Acad. Sci. USA.* 98:11277–11282. http://dx.doi.org/10.1073/pnas.201250598

Cox, D.H., J. Cui, and R.W. Aldrich. 1997. Allosteric gating of a large conductance Ca-activated K^+ channel. *J. Gen. Physiol.* 110:257–281. http://dx.doi.org/10.1085/jgp.110.3.257

Decher, N., J. Chen, and M.C. Sanguinetti. 2004. Voltage-dependent gating of hyperpolarization-activated, cyclic nucleotide-gated pacemaker channels: molecular coupling between the S4-S5 and C-linkers. *J. Biol. Chem.* 279:13859–13865. http://dx.doi.org/10.1074/jbc.M313704200

DiFrancesco, D., and P. Tortora. 1991. Direct activation of cardiac pacemaker channels by intracellular cyclic AMP. *Nature.* 351:145–147.

Doyle, D.A., J. Morais Cabral, R.A. Pfuetzner, A. Kuo, J.M. Gulbis, S.L. Cohen, B.T. Chait, and R. MacKinnon. 1998. The structure of the potassium channel: molecular basis of K+ conduction and selectivity. *Science.* 280:69–77.

Gagnon, D.G., and F. Bezanilla. 2009. A single charged voltage sensor is capable of gating the Shaker K^+ channel. *J. Gen. Physiol.* 133:467–483. http://dx.doi.org/10.1085/jgp.200810082

Gauss, R., R. Seifert, and U.B. Kaupp. 1998. Molecular identification of a hyperpolarization-activated channel in sea urchin sperm. *Nature.* 393:583–587. http://dx.doi.org/10.1038/31248

Hille, B. 2001. Ion channels of excitable membranes. Sinauer Associates, Inc., Sunderland, MA. 814 pp.

Holmgren, M., K.S. Shin, and G. Yellen. 1998. The activation gate of a voltage-gated K+ channel can be trapped in the open state by an intersubunit metal bridge. *Neuron.* 21:617–621. http://dx.doi.org/10.1016/S0896-6273(00)80571-1

Horrigan, F.T., and R.W. Aldrich. 2002. Coupling between voltage sensor activation, Ca^{2+} binding and channel opening in large conductance (BK) potassium channels. *J. Gen. Physiol.* 120:267–305. http://dx.doi.org/10.1085/jgp.20028605

Islas, L.D., and F.J. Sigworth. 1999. Voltage sensitivity and gating charge in *Shaker* and *Shab* family potassium channels. *J. Gen. Physiol.* 114:723–742. http://dx.doi.org/10.1085/jgp.114.5.723

Kaupp, U.B., T. Niidome, T. Tanabe, S. Terada, W. Bönigk, W. Stühmer, N.J. Cook, K. Kangawa, H. Matsuo, T. Hirose, et al. 1989. Primary structure and functional expression from complementary DNA of the rod photoreceptor cyclic GMP-gated channel. *Nature.* 342:762–766. http://dx.doi.org/10.1038/342762a0

Kwan, D.C., D.L. Prole, and G. Yellen. 2012. Structural changes during HCN channel gating defined by high affinity metal bridges. *J. Gen. Physiol.* 140:279–291. http://dx.doi.org/10.1085/jgp.201210838

Larsson, H.P., O.S. Baker, D.S. Dhillon, and E.Y. Isacoff. 1996. Transmembrane movement of the shaker K+ channel S4. *Neuron.* 16:387–397. http://dx.doi.org/10.1016/S0896-6273(00)80056-2

Liu, Y., M. Holmgren, M.E. Jurman, and G. Yellen. 1997. Gated access to the pore of a voltage-dependent K+ channel. *Neuron.* 19:175–184. http://dx.doi.org/10.1016/S0896-6273(00)80357-8

Long, S.B., E.B. Campbell, and R. Mackinnon. 2005. Crystal structure of a mammalian voltage-dependent Shaker family K+ channel. *Science.* 309:897–903. http://dx.doi.org/10.1126/science.1116269

Lu, Z., A.M. Klem, and Y. Ramu. 2002. Coupling between voltage sensors and activation gate in voltage-gated K^+ channels. *J. Gen. Physiol.* 120:663–676. http://dx.doi.org/10.1085/jgp.20028696

Ludwig, A., X. Zong, M. Jeglitsch, F. Hofmann, and M. Biel. 1998. A family of hyperpolarization-activated mammalian cation channels. *Nature.* 393:587–591. http://dx.doi.org/10.1038/31255

Männikkö, R., F. Elinder, and H.P. Larsson. 2002. Voltage-sensing mechanism is conserved among ion channels gated by opposite voltages. *Nature.* 419:837–841. http://dx.doi.org/10.1038/nature01038

Mayer, M.L., and G.L. Westbrook. 1983. A voltage-clamp analysis of inward (anomalous) rectification in mouse spinal sensory ganglion neurones. *J. Physiol.* 340:19–45.

Pathak, M.M., V. Yarov-Yarovoy, G. Agarwal, B. Roux, P. Barth, S. Kohout, F. Tombola, and E.Y. Isacoff. 2007. Closing in on the resting state of the Shaker K(+) channel. *Neuron.* 56:124–140. http://dx.doi.org/10.1016/j.neuron.2007.09.023

Prole, D.L., and G. Yellen. 2006. Reversal of HCN channel voltage dependence via bridging of the S4–S5 linker and Post-S6. *J. Gen. Physiol.* 128:273–282. http://dx.doi.org/10.1085/jgp.200609590

Rothberg, B.S., K.S. Shin, P.S. Phale, and G. Yellen. 2002. Voltage-controlled gating at the intracellular entrance to a hyperpolarization-activated cation channel. *J. Gen. Physiol.* 119:83–91. http://dx.doi.org/10.1085/jgp.119.1.83

Rothberg, B.S., K.S. Shin, and G. Yellen. 2003. Movements near the gate of a hyperpolarization-activated cation channel. *J. Gen. Physiol.* 122:501–510. http://dx.doi.org/10.1085/jgp.200308928

Ryu, S., and G. Yellen. 2012. Charge movement in gating-locked HCN channels reveals weak coupling of voltage sensors and gate. *J. Gen. Physiol.* 140:469–479.

Santoro, B., D.T. Liu, H. Yao, D. Bartsch, E.R. Kandel, S.A. Siegelbaum, and G.R. Tibbs. 1998. Identification of a gene encoding a hyperpolarization-activated pacemaker channel of brain. *Cell.* 93:717–729. http://dx.doi.org/10.1016/S0092-8674(00)81434-8

Shin, K.S., C. Maertens, C. Proenza, B.S. Rothberg, and G. Yellen. 2004. Inactivation in HCN channels results from reclosure of the activation gate: desensitization to voltage. *Neuron.* 41:737–744. http://dx.doi.org/10.1016/S0896-6273(04)00083-2

Tristani-Firouzi, M., J. Chen, and M.C. Sanguinetti. 2002. Interactions between S4-S5 linker and S6 transmembrane domain modulate gating of HERG K+ channels. *J. Biol. Chem.* 277:18994–19000. http://dx.doi.org/10.1074/jbc.M200410200

Wang, J., S. Chen, M.F. Nolan, and S.A. Siegelbaum. 2002. Activity-dependent regulation of HCN pacemaker channels by cyclic AMP: signaling through dynamic allosteric coupling. *Neuron.* 36:451–461. http://dx.doi.org/10.1016/S0896-6273(02)00968-6

Warmke, J.W., and B. Ganetzky. 1994. A family of potassium channel genes related to eag in Drosophila and mammals. *Proc. Natl. Acad. Sci. USA.* 91:3438–3442. http://dx.doi.org/10.1073/pnas.91.8.3438

Zagotta, W.N., T. Hoshi, and R.W. Aldrich. 1994. Shaker potassium channel gating. III: Evaluation of kinetic models for activation. *J. Gen. Physiol.* 103:321–362. http://dx.doi.org/10.1085/jgp.103.2.321

Zagotta, W.N., N.B. Olivier, K.D. Black, E.C. Young, R. Olson, and E. Gouaux. 2003. Structural basis for modulation and agonist specificity of HCN pacemaker channels. *Nature.* 425:200–205. http://dx.doi.org/10.1038/nature01922