What activates inactivation?

Christopher A. Ahern

Department of Molecular Physiology and Biophysics, The University of Iowa, Iowa City, IA 52242

Voltage-gated sodium channels play a central role in action potential firing throughout the cardiovascular and nervous systems, and their gating is exquisitely sensitive to changes in transmembrane potential. Negative regulation of sodium channel conductance occurs through a process known as inactivation, which can proceed from either the open or closed states, termed "fast" or "steady-state" inactivation (SSI), respectively. When sodium channel conductance is poorly regulated, very bad things happen. For instance, inherited or acquired defects in sodium channel conductance are associated with a spectrum of electrical signaling disorders including cardiac arrhythmias (Wang et al., 1995; Valdivia et al., 2005), epilepsy and primary erythermalgia (a peripheral pain disorder) (Yang et al., 2004), paroxysmal extreme pain disorder (Fertleman et al., 2006), hypokalemic periodic paralysis (Ptácek et al., 1991; Rojas et al., 1991), paramyotonia congenital (McClatchey et al., 1992), in addition to unexpected roles in migraine (Kahlig et al., 2008), autism (Weiss et al., 2003; Han et al., 2012a), sleep (Han et al., 2012b), and multiple sclerosis (Craner et al., 2004). Furthermore, SSI strongly influences electrical stability in excitable cells because the midpoint of the inactivation–voltage relationship is often near the resting membrane potential of the cell; thus, seemingly modest shifts in the midpoint of the SSI versus voltage relationship, caused by (dys)modulation or point mutations, can have a powerful effect on the number of channels that are available to contribute to the action potential. Thus, sodium channel gating, and inactivation in particular, is a biophysical phenomenon that effortlessly transcends the patch rig to the clinical setting, yet a detailed picture of the molecular basis that underlies inactivation remains stubbornly unresolved. In this issue of *The Journal of General Physiology*, Capes et al. used a voltage sensor–disabling approach to systematically investigate the identity of the molecular trigger for inactivation and confirm the role for the domain four (DIV S4) voltage sensor in this key physiological process (Capes et al., 2013).

Rapid sodium channel activation drives the upstroke of the action potential, but fast and complete inactivation of sodium conductance is essential for timely membrane repolarization and the refractory interval between action potentials. Hodgkin and Huxley presciently described a mechanism whereby four membrane-embedded charged particles, three associated with activation (m-gates) and one with inactivation (h-gate), give rise to the voltage-dependent sodium conductance in squid axon (Hodgkin and Huxley, 1952). This idea is consistent with the sodium channel gene structure that contains four nonidentical domains (DI–DIV), each with pore-lining S5–p-loop–S6 segments and a voltage-sensing domain (VSD) comprised of the S1–S4 segments, with the S4 segments harboring between three and seven positively charged residues, depending on the domain. Mutations throughout the channel can affect gating; however, those introduced in DIV tend to most strongly affect inactivation (Chahine et al., 1994; McPhee et al., 1994, 1998; Chen et al., 1996; Yang et al., 1996; Lerche et al., 1997). The fast kinetics of DI–III S4 movement, as visualized by voltage-clamp fluorometry, correlate closely with activation of sodium conductance, whereas the relatively slow movement of DIV S4 aligns with the development of inactivation and with the immobilization of the gating charge (Cha et al., 1999; Chanda and Bezanilla, 2002). Furthermore, toxins that preferentially interact with the DIV VSD potently modulate channel inactivation (Hanck and Sheets, 2007; Bosmans et al., 2008; Wang et al., 2011). Thus, a plethora of evidence supports the idea that DI–III contribute to channel activation and DIV S4 is associated with inactivation. However, it is not known whether DIV S4 activation alone is sufficient to initiate inactivation, and if this single trigger is responsible for allowing inactivation to proceed from both open and closed channels.

To tackle this question directly, Capes et al. (2013) used a charge neutralization strategy whereby the first three S4 charges, which carry the bulk of the charge movement (Sheets et al., 1999), were mutated to glutamine (Q), resulting in charge-neutral (CN) voltage sensors. In addition to impairing S4 voltage sensitivity and movement, such CN VSDs are likely to be in an active conformation, which is usually only visited at positive potentials (Bao et al., 1999; Gagnon and Bezanilla,

Correspondence to Christopher A. Ahern: christopher-ahern@uiowa.edu

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2009; Capes et al., 2012). In the present case, these mutations were made individually in each of the four S4 segments of skeletal muscle sodium channels, and the resulting channels were electrophysiologically interrogated for activation and inactivation characteristics. All four CN sodium channels were functional and had robust voltage-dependent activation gating, which at first glance is surprising, given that the S4 segments had been electrostatically neutered. However, if S4 neutralization promotes movement of the S4 segment into the activated conformation, one has in fact removed an energy barrier to activation, explaining the "normal" conductance–voltage relationships of VSD-disabled channels. In terms of inactivation, DI–III CN channels were again quite functionally tolerant, whereas DIV CN channels have altered inactivation properties from closed, open, and inactivated states. First, DIV CN channels displayed a large hyperpolarizing shift in the SSI midpoint, suggesting that they were "preinactivated" at negative potentials, consistent with the hypothesis that DIV S4 activation is sufficient for SSI and that the mutation "preactivates" the DIV S4 segment. Second, DIV CN channels also showed an accelerated and near instantaneous entry into fast-inactivated states as determined by a two-pulse protocol to avoid the contribution of activation latencies (Aldrich et al., 1983). Third, once inactivated, DIV CN channels lagged in leaving nonconducting states, and once initiated, the recovery from inactivation was significantly slowed. Fourth, all three of these experimental results could be recapitulated by a model of sodium channel gating whereby inactivation, from either open or closed states, is initiated by DIV S4 movement, after which an inactivation particle may bind through a weakly voltage-dependent step. Despite the wrinkle that the QQQ triplet mutation may functionally affect each S4 segment differently, the data produced a clear result and, together with previous work, support the notion that, although all four voltage sensors activate in channel opening, DIV S4 activation alone is sufficient for initiation of both fast and SSI, as depicted in Fig. 1. For simplicity, the stochastic activation of the DI–DIII VDSs are combined as a single step that ends with channel opening, as shown on the pathway to the right-hand side. The subsequent activation of DIV VSD results in additional pore conformations (Goldschen-Ohm et al., 2013), and the eventual activation

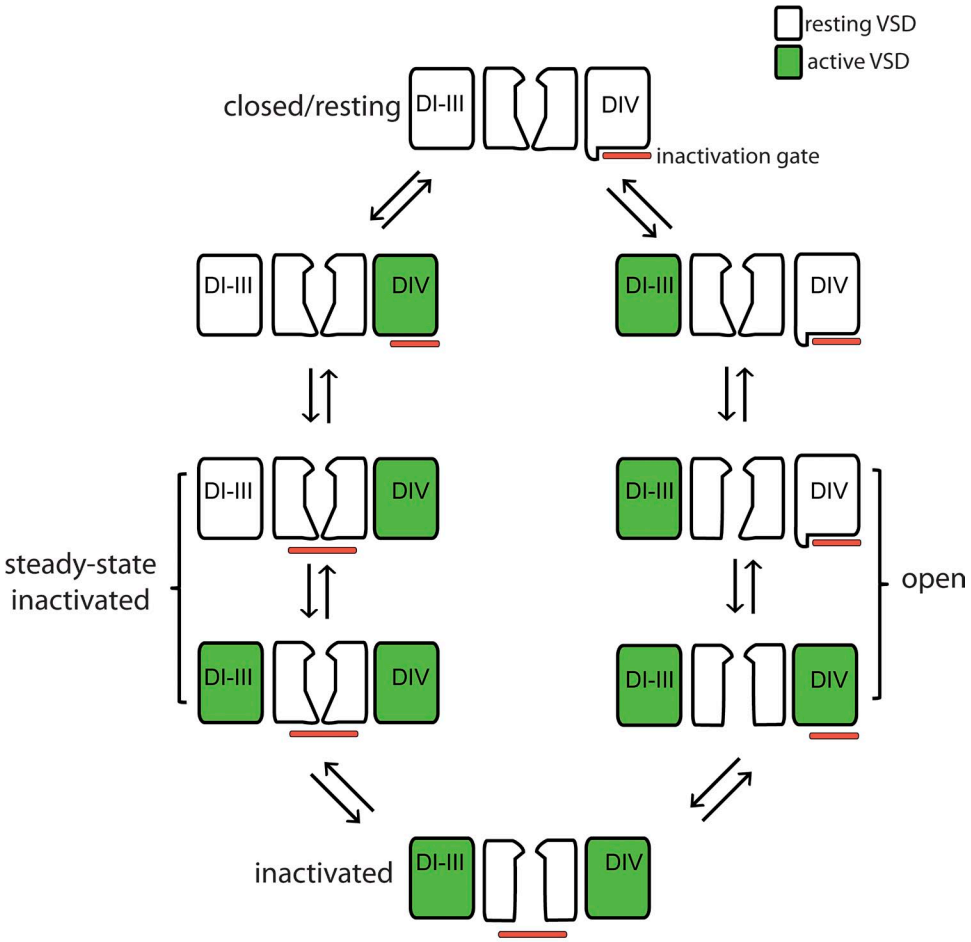

Figure 1. The life cycle of sodium channel inactivation. (Top) A simplified model of a voltage-gated sodium channel, with the DI–DIII voltage sensors functionally compartmentalized from DIV and an inactivation "gate" (red bar) that is held in place by the DIV VSD. (Right) The potential contributions of DI–III to activation and the DIV VSD to fast inactivation from the open conformation. DIV S4 activation (bottom right) allows for the inactivation gate to relocate to a pore site, occluding sodium conductance. (Left) SSI proceeds after DIV activation through a series of nonconducting states. The possibility of a single inactivated conformational end point with all VSDs activated is shown at the bottom, consistent with the kinetic scheme in Fig. 6 of Capes et al. (2013) in this issue of the Journal.

of fast inactivation, bottom right. Speculative domain contributions involved with SSI portrayed on the left are described in the legend and flow through a series of electrically silent conformations (Horn et al., 1981). Does inactivation from open or closed states produce a common nonconducting conformation? One untested but compelling possibility is that DIV S4 activation promotes a pore conformation that is permissive to inactivation through the binding of regions of the channel, such as the DIII–IV linker triplet of residues IFM, which have been shown to disrupt inactivation when mutated (West et al., 1992). In the case of closed-state inactivation, DIV S4 activation and subsequent conformations would be electrically silent but may still share a similar inactivated conformation with fast-inactivated channels. However, it is also possible that, as in voltage-gated potassium channels, distinct pore regions are used for different types of inactivation (Choi et al., 1991). Although the data are consistent with the notion that DIV S4 represents a single molecular switch for closed- and open-state inactivation, little molecular detail is available on the transient complexes formed between DIV S4 movement and the development of inactivation or the location(s) of putative pore regions that might serve as a receptor for an inactivation particle. Indeed, given the many mechanistic unknowns in regards to sodium channel inactivation, the paper by Capes et al. (2013), like DIV S4 activation, is just the beginning of the story.

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