What makes a sodium channel?

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Action potentials in mammalian nerve and muscle are carried by sodium currents through voltage-gated sodium channels (Na_V). These proteins are part of the larger family of voltage-gated channels that includes the well-known calcium (Ca_V) and potassium (K_V) channels, as well as a variety of other channel types, including Catsper, two-pore channels, and bacterial sodium channels ($BacNa_V$). They even have distant homology to the IP3/ryanodine receptor, ionotropic glutamate receptor, and voltage-sensitive phosphatase families. However, it now seems that the large family of canonical Na_V—the monophyletic group that includes all of the animal channels with typical Hodgkin-Huxley–like sodium currents—is a house divided: many appear to be selective for calcium. In this issue, Gosselin-Badaroudine et al. (2016. *J. Gen. Physiol.* http://dx.doi.org/10.1085/jgp.201611614) describe a novel channel from the honeybee *Apis mellifera* that is the most calcium-selective Na_V family member to date.

One of the most interesting advances in recent years is that channels sharing the description "voltage gated" and "sodium channel" are not necessarily part of the same family, evolutionarily speaking. Instead, voltage-gated sodium channels seem to have evolved many times independently of one another, often within vastly different families of channels. These include bacterial channels that are only distantly related to eukaryotic Na_Vs (Ren et al., 2001; Liebeskind et al., 2013), intracellular two-pore channels (Wang et al., 2012), and even T-type Ca_Vs (the latter being the result of a single splicing event; Senatore et al., 2014). One interesting observation is that many of these families have arisen from calcium channels (Liebeskind et al., 2012, 2013; Moran et al., 2015), confirming Hille's assertion that canonical Na_Vs arose from Ca_Vs (Hille, 1989) and extending it to other families. It seems that evolution has played the same trick time and time again, turning Ca_Vs into Na_Vs.

Even in the canonical Na_V family, many members are likely to be calcium-selective channels (Zhou et al., 2004; Liebeskind et al., 2011; Gur Barzilai et al., 2012). Sodium selectivity within this family arose twice independently: once in vertebrates and once in cnidarians, such as jellyfish and sea anemones (Gur Barzilai et al., 2012). Thus, there exists a hidden world of channels that are phylogenetically Na_V channels but biophysically Ca_V channels. These Na_V/Ca_V channels have not been described until recently, perhaps because well-studied vertebrates have retained only the strictly Na_V type (Liebeskind et al., 2011). Invertebrates, which form the vast majority of animal species, have kept the Na_V/Ca_V type, and thus a major aspect of animal nervous systems, and a key part of our own history, has remained in the dark.

In this issue, Gosselin-Badaroudine et al. provide the most thorough description of a Na_V/Ca_V channel to date. Like others of its kind, the honeybee channel has a DEEA selectivity filter motif, intermediate, as it were, between the canonical Na_V motif (DEKA) and the canonical Ca_V motifs (EEEE or EEDD). It also has much slower kinetics than the Hodgkin-Huxley–type Na_V channels, operating on the scale of hundreds of milliseconds. The channel expresses robustly in *Xenopus laevis* oocytes, making it a prime candidate for a model Na_V/Ca_V channel.

But what makes this channel really interesting is that, unlike previously described Na_V/Ca_V channels from cockroaches and sea anemones (Zhou et al., 2004; Gur Barzilai et al., 2012) that can pass both ions, it appears to have little or no sodium permeability. Sodium permeability was achieved by the authors after a single E→K mutation in the third domain, but even with this mutation, calcium still partially blocked conductance. Intriguingly, the block appeared to be insufficient to produce the anomalous mole fraction effect (AMFE) that typical Ca_V channels exhibit in mixed ionic solutions. This is an important difference. The classical view, put forward by Hille and others, is that sodium selectivity arose from calcium selectivity by removal of the high-affinity binding site for divalents, the site that gives rise to the AMFE in canonical calcium channels and allows them to "pluck rare calcium ions out of a sea of sodium" (Hille, 2001). Does the honeybee channel represent an intermediate state where the high-affinity site is partially removed? Does it represent a completely different mode of calcium selectivity? Does it select for the rare Ca^{2+} ions without a high-affinity site? Or does it have a high-affinity site that, perhaps because of a unique pore geometry, does not give rise to an AMFE? This new channel raises a bevy

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of interesting questions that impinge directly on our understanding of how vertebrate sodium channels choose between these two crucial ions.

Because the honeybee channel possesses the same selectivity filter as the recently described cockroach and sea anemone Na_V/Ca_V channels but has a different selectivity profile, it naturally leads one to suspect the importance of pore residues outside the canonical selectivity filter motif. Indeed, Gur Barzilai et al. (2012) found that altering the selectivity filter of Nematostella's Na_V/Ca_V from DEEA to the canonical DEKA was not enough to reach normal (i.e., vertebrate) levels of selectivity for sodium over potassium. Furthermore, they found that reconstruction of the DKEA selectivity motif from the Nematostella sodium-selective variant in the background of the DEEA-bearing variant resulted in a nonselective channel, but putting in the entire pore loops of the DKEA channel resulted in a bona fide sodium channel. Thus, it appears that channels in this family rely on more than just the canonical four amino acids for selectivity.

But that's not all. Gosselin-Badaroudine et al. (2016) found that calcium permeation altered the honeybee channel's kinetics. Na_V/Ca_V pores are therefore rich with mostly unexplored functional properties that seem to vary on a fairly rapid evolutionary timescale (e.g., between honeybees and cockroaches). Unfortunately, there are no high-resolution structures of four-domain, eukaryotic sodium channels; only distantly related bacterial channels with symmetrical or pseudosymmetrical pore architecture have been solved crystallographically (Payandeh and Minor, 2015). The evolutionary record therefore remains a key resource for researchers interested in the principles of sodium selectivity. As long as we are willing to look outside typical model systems, we will continue to find interesting variants that challenge our notions of selectivity and other important functions. So whether one defines these mysterious channels phylogenetically (Na_V2; Liebeskind et al., 2011), functionally (Ca_V4, as the present authors do), or ambiguously (Na_V/Ca_V), this new channel begs, and may help to answer, the question, what makes a sodium channel?

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