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Voltage-gated sodium channels viewed through a structural biology lens

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Voltage-gated sodium (Nav) channels initiate and propagate action potentials in excitable cells, and are frequently dysregulated or mutated in human disease. Despite decades of intense physiological and biophysical research, eukaryotic Nav channels have so far eluded high-resolution structure determination because of their biochemical complexity. Recently, simpler bacterial voltage-gated sodium (BacNav) channels have provided templates to understand the structural basis of voltage-dependent activation, inactivation, ion selectivity, and drug block in eukaryotic Nav and related voltage-gated calcium (Cav) channels. Further breakthroughs employing BacNav channels have also enabled visualization of bound small molecule modulators that can guide the rational design of next generation therapeutics. This review will highlight the emerging structural biology of BacNav channels and its contribution to our understanding of the gating, ion selectivity, and pharmacological regulation of eukaryotic Nav (and Cav) channels.

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Introduction

Voltage-gated sodium (Nav), calcium (Cav), and potassium (Kv) channels form a superfamily of ion channels that open and close ion-selective pores in response to small changes in membrane potential [1,2]. High-resolution structures of homotetrameric K^+ channels have been available for nearly two decades [3••,4••,5••,6••], but eukaryotic Nav and Cav channels lack the structural four-fold symmetry found in Kv channels [7,8], which has important implications for understanding channel gating and ion selectivity [9]. Furthermore, whereas Kv channels function to return depolarized neurons to their resting state, eukaryotic Nav channels are highly specialized for their role in conducting the ionic currents that initiate the rising phase of action potentials. Hallmark Nav channel characteristics include rapid voltage-dependent activation, sodium selectivity, and mechanisms of fast and slow inactivation [1,9]. Mutations in the nine Nav channel isoforms differentially expressed throughout the human body are associated with migraine (Nav1.1), epilepsy (Nav1.1-1.3, Nav1.6), pain (Nav1.7-1.9), cardiac arrhythmias (Nav1.5), and muscle paralysis syndromes (Nav1.4) [10–12]. In addition to being therapeutic targets for the treatment of various neurological, cardiovascular, and muscular disorders [12–14], Nav channels are also targeted by numerous environmental toxins [12,15,16]. Nav channel inhibitors currently used in the clinic all bind within the central cavity of the ion pore and lack molecular selectivity [17,18]. This is due to the high sequence conservation among channel isoforms and limits the therapeutic utility of available drugs.

Eukaryotic Nav channels contain a large pore-forming α-subunit (~2000 residues) that associates with auxiliary subunits and undergoes extensive post-translational modifications [9,19], including *N*-linked glycosylation and potential phosphorylation, ubiquitination, arginine methylation, palmitylation, sulfation and *S*-nitrosylation [20]. This biochemical complexity has so far frustrated crystallographic studies and structure-based drug design efforts. Fundamental questions about the structural basis of channel activation, ion selectivity, inactivation, and modulation therefore also remain unanswered. The impact of a wide spectrum of inherited and spontaneous disease mutations on Nav channel function are poorly understood [21,22], and further highlight the need to obtain high-resolution views of these channels.

An important breakthrough on the road to structural elucidation was realized with the first electrophysiological characterization of a Nav channel cloned from bacteria. This so-called NaChBac channel was found to share key functional and pharmacological properties with its eukaryotic Nav and Cav channel relatives [23••,24–29]. Hundreds of bacterial Nav (BacNav) channels have since been identified [28,30,31] and are thought to play a role in bacterial motility and ionic homeostasis [32–34]. BacNav channels are simple homotetramers of ~250 amino acids that lack auxiliary

Table 1

Summary of BacNav channel structures determined to date. For publications with multiple PDB entries only a representative PDB code is listed

Channel	Organism	PDB ID	Resolution	Year	Notable Structural Elements	References
NavAb	*Arcobacter butzleri*	3RVY	2.7 Å	2011	First BacNav structure, proposed as 'pre-open' state	[35••]
NavAb	*Arcobacter butzleri*	4EKW	3.2 Å	2012	Collapsed selectivity filter, asymmetric pore, proposed as inactivated state	[36••]
NavRh	*Rickettsiales sp. HIMB114*	4DXW	3.1 Å	2012	Ca^{2+} blocked selectivity filter, asymmetric pore, proposed as inactivated state	[40••]
NavMs	*Magnetococcus marinus*	4F4L	3.5 Å	2012	First pore-only structure, proposed open pore model	[41•]
NavCt	*Caldalkalibacillus thermarum*	4BGN	9-10 Å	2013	Electron crystallography, S6 activation gate seen in two conformations	[46•]
NavAe1	*Alkalilimnicola ehrlichii*	4LTO	3.5 Å	2014	Pore-only structure with C-terminal coiled-coil resolved	[44••]
NavMs	*Magnetococcus marinus*	4PA4	3.0 Å	2014	Pore-only structure with bound pore blocker modeled	[42••]
CavAb	*Arcobacter butzleri*	2MS2	2.8 Å	2014	Structures of a model Ca^{2+} selectivity filter	[37••]
Nav1.7-NavAb	*Homo sapiens-A.butzleri chimera*	5EK0	3.5 Å	2015	Human Nav1.7 VSD4-NavAb chimera with a bound antagonist	[38••]
NavMs	*Magnetococcus marinus*	5BZB	2.7 Å	2016	Pore-only structure with Na^+ assigned in the selectivity filter	[43•]
NavAe1	*Alkalilimnicola ehrlichii*	5HK7	3.0 Å	2016	Pore-only structures with mutant C-terminal 'neck' domains	[45••]
CavAb	*Arcobacter butzleri*	5KLG	3.3 Å	2016	Model Cav channel with antagonists bound	[39••]

subunits and post-translational modifications. Several have proven amenable to crystallization and experimental structures have now been reported for the NavAb [35••,36••,37••,38••,39••], NavRh [40••], NavMs [41•,42••,43•], NavAe1 [44••,45••], and NavCt [46•] channels (Table 1). As we summarize below, these foundational studies provide first glimpses into the structural underpinnings of voltage-dependent activation, inactivation, ion selectivity, lipid modulation, and drug block across the Nav and Cav channel superfamily.

General architecture of BacNav channels

Canonical voltage-gated ion channels contain six transmembrane segments (S1–S6) as their basic structural unit [1,2], and are typically assembled as homotetramers in BacNav and Kv channels (Figures 1a–c) [6,35••,40••,47••]. In eukaryotic Nav and Cav channels, four units are linked into a single polypeptide [7,8], allowing for functional specialization of their four homologous but non-identical S1–S6 domains (Figure 1a) [9,48]. The full-length crystal structures of NavAb and NavRh established that the first four transmembrane segments (S1–S4) form peripheral voltage sensor domains (VSDs), while the last two segments (S5–S6) assemble the central ion-conducting pore module (PM) (Figure 1c) [35••,40••], corroborating early Kv channel structures [47••].

The BacNav pore module

The selectivity filter of the BacNav channel PM is scaffolded by the P1 (pore 1) and P2 (pore 2) helices, which form a critical helix-loop-helix motif (Figures 1a–c, 2a) [35••]. The P2-helix lines an electronegative extracellular vestibule that attracts cations towards the pore (Figure 2b); this conserved structural feature may also play a role in binding blocking ions and toxins (e.g. tetrodotoxin) at the extracellular mouth of the selectivity filter in eukaryotic channels [44••,49–51]. In NavAb and NavMs, the selectivity filter signature sequence of TLESWS is housed in-between the P1 and P2-helices, where it forms a ~5 Å wide ion conduction pathway (Figures 2a–c) [35••,43•]. Four glutamic acid side-chains (TL**E**SWS) directly line the filter entryway and participate in substrate selection, followed by two concentric rings of backbone carbonyls (**TL**ESWS) that promote the passage of hydrated sodium ions (Figure 2c). In contrast to the constrained, dehydrating selectivity filters found in K^+ channels [4••,52], ion conduction appears to occur through a weak or loosely coupled knock-on mechanism within a selectivity filter that prefers passing hydrated Na^+ ions over hydrated K^+ or Ca^{2+} ions [43•,53–56]. Remarkably, the NavRh channel has a divergent selectivity filter sequence (TLSSW**E**) that appears to remain selective for Na^+ ions [40••,57]; together with the asymmetrically arranged signature 'DEKA' selectivity filter motif found in canonical eukaryotic Nav channels [7,58,59], this indicates that nature has found distinct molecular routes to establish Na^+-selective pores on related structural scaffolds [9,60].

Analogous to eukaryotic Nav channels [61], BacNav channels can be converted into highly selective Ca^{2+} channels through simple mutation of the selectivity filter sequence (e.g. TL**DD**WS**N** in the case of NavAb)