Review

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Deciphering voltage-gated Na^+ and Ca^{2+} channels by studying prokaryotic ancestors

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Voltage-gated sodium channels (Na_Vs) and calcium channels (Ca_Vs) are involved in electrical signaling, contraction, secretion, synaptic transmission, and other physiological processes activated in response to depolarization. Despite their physiological importance, the structures of these closely related proteins have remained elusive because of their size and complexity. Bacterial Na_Vs have structures analogous to a single domain of eukaryotic Na_Vs and Ca_Vs and are their likely evolutionary ancestor. Here we review recent work that has led to new understanding of Na_Vs and Ca_Vs through high-resolution structural studies of their prokaryotic ancestors. New insights into their voltage-dependent activation and inactivation, ion conductance, and ion selectivity provide realistic structural models for the function of these complex membrane proteins at the atomic level.

Na_Vs and Ca_Vs and their bacterial ancestors

Na_Vs initiate action potentials in excitable cells and are crucial for electrical signaling from bacteria to humans [1]. Ca_Vs are activated by depolarization during action potentials and Ca^{2+} entry through them initiates synaptic transmission, muscle contraction, hormone secretion, and many other biochemical and physiological processes [2,3]. These channels are thought to share similar voltage-dependent activation and inactivation processes whose structural basis is fundamental for electrical signaling. Moreover, how these channels can rapidly and selectively conduct Na^+ or Ca^{2+} ions in response to changes of the electrical membrane potential is a crucial question in biology.

Mammalian Na_Vs are complexes of a large α subunit of 260 kDa and smaller β subunits of 30–40 kDa [4]. cDNA encoding the pore-forming α subunits is sufficient for expression of functional Na_Vs, whereas the β subunits enhance expression, modulate Na_V gating, and serve as cell adhesion molecules (reviewed in [5,6]). Na_V α subunits are polypeptides of approximately 2000 amino acid

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Glossary

Activation gate: a structural element at the intracellular end of a voltage-gated ion channel that controls the opening and closure of the pore in response to VSM activation.
Activated state: the state of a Na_V or Ca_V during membrane depolarization. The channel is characterized by an open pore and the S4 segments of the VSMs in outward, activated positions.
Depolarization: a rapid shift of the charge inside the cell from negative to neutral or positive.
Fast inactivation: rapid inactivation of eukaryotic Na_Vs within 1–2 ms. The inactivation process involves a hinged-lid mechanism in which the pore is plugged by the intracellular linker between domains III and IV. This structural feature is not present in bacterial Na_Vs. Fast inactivation is rapidly reversed with a few milliseconds on repolarization.
Gating charges: positively charged amino acid residues (usually Arg) located at three-residue intervals along the S4 transmembrane segment in the VSM. The electrical field caused by the membrane potential exerts an electrostatic force on these positive charges. At the negative internal resting membrane potential, these positive gating charges are pulled inward like the trigger of a cocked gun. Depolarization releases this electrostatic force, allowing the gating charges and the S4 segment to move outward rapidly, activate the VSM, and initiate the conformational changes that open the pore. Repolarization pulls the gating charges back into their inward, resting position where they are ready for activation again on depolarization.
Inactivated state: the state adopted by a Na_V or Ca_V during prolonged depolarization. Inactivated states are characterized by one or more VSMs in activated conformation but a blocked pore.
'Knock-off' mechanism: a hypothetical ion conductance mechanism in which ionic repulsion triggered by simultaneous occupation of adjacent ion-binding sites facilitates the rapid and unidirectional movement of ions through the selectivity filter.
Pre-open state: a transitional state of a Na_V or Ca_V under membrane depolarization. This state is characterized by activated VSMs with the S4 helix in an outward, activated position with the pore still closed but poised to spring open.
Pore module: the S5 and S6 transmembrane segments and intervening P loop that form the central pore of a voltage-gated ion channel where permeating ions are conducted.
Resting state: the state of a Na_V or Ca_V under resting membrane potential. The resting state of the channel is characterized by a closed pore with low open probability and the S4 segments of the VSMs in inward, resting positions.
Selectivity filter: a constriction of an ion channel in the pore that confers selective permeation of specific ions by the channel.
Slow inactivation: inactivation of both prokaryotic and eukaryotic Na_Vs over hundreds of milliseconds. The inactivation mechanism involves rearrangement of the pore. Slow inactivation is slowly reversed on repolarization. Voltage-dependent inactivation of eukaryotic Ca_Vs is likely to share a similar mechanistic and structural basis.
Stepwise permeation: a rate theory-based ion permeation mechanism for Ca_Vs in which stepwise changes in the binding affinities of Ca^{2+} ions to multiple sites of the selectivity filter facilitate the high Ca^{2+} flux without repulsive interactions. The energy barriers are lower, and therefore conduction rates faster, for stepwise movement from free solution to high-affinity ion-binding sites than for a single jump from free solution to a high-affinity ion-binding site.
Voltage-sensing module: a four-transmembrane helical module that confers voltage sensitivity on voltage-gated ion channels.

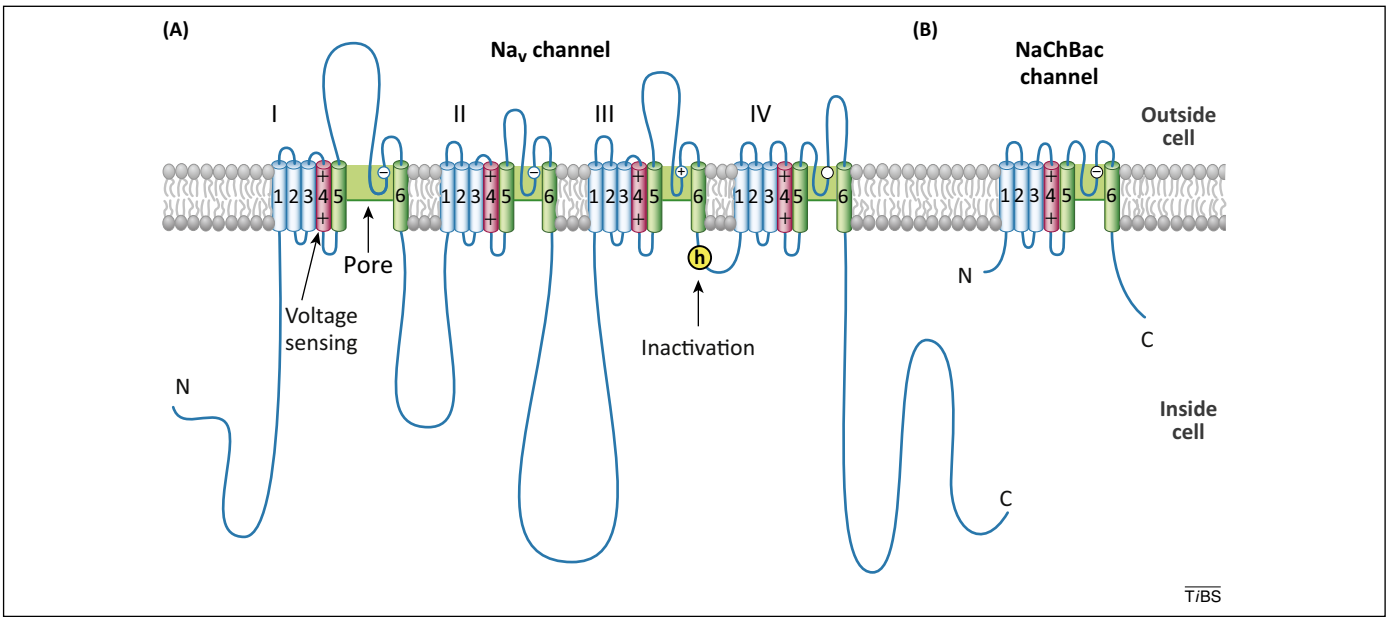

Figure 1. Voltage-gated sodium channel (Na_V) structure. (**A**) 2D schematic map of Na_V structure and function. The α subunit of $Na_V1.2$ is illustrated as a transmembrane folding diagram in which cylinders represent transmembrane α helices and lines represent connecting amino acid sequences in proportion to their length. The Roman numerals indicate the four homologous domains and the Arabic numerals are used to label the six transmembrane helices. The S4 helices are colored in red with '+' signs indicating gating charges. The S5–S6 helices are colored in green and the small white circles indicate key residues in the selectivity filter with '+' and '–' signs indicating their charge states. The yellow circle with an 'h' indicates the inactivation gate. (**B**) Schematic map of the bacterial NaChBac channel, which contains the minimal functional elements of a single homologous domain in a mammalian Na_V.

residues organized into four homologous domains, each containing six transmembrane segments (Figure 1A) [5,6]). Each homologous domain comprises two functional modules: a voltage-sensing module (VSM) comprising the S1–S4 segments and a pore-forming module (PM) comprising the S5 and S6 segments and the P loop between them (Figure 1A).

Mammalian Ca_Vs are also multisubunit complexes [3]. They have a central pore-forming α1 subunit that is analogous in structure and function to the Na_V α subunit. The α1 subunit is associated with an intracellular β subunit, a membrane-associated α2δ disulfide-linked complex, and a transmembrane γ subunit, all of which are involved in channel regulation but not in voltage-dependent gating or ion conductance. Analysis of the pore-forming sequences of the 143 voltage-gated ion channels and their relatives in the human genome predicted a common core domain composition with many key conserved structural elements. These conserved features led to the proposal that Na_Vs and Ca_Vs derive from a common ancestor and have a similar structural basis for their function [7]. Despite their high biological significance, the sheer size and complex transmembrane architecture of these channel proteins have posed a major challenge for structural and mechanistic analyses of their functions.

The unexpected discovery of the bacterial Na_V NaChBac was a landmark in ion channel research [8]. The sequence of NaChBac is analogous to one domain of the eukaryotic Na_V or Ca_V and functions as a homotetramer (Figure 1B) [8]. Orthologs of NaChBac are found in Gram-positive and Gram-negative eubacteria and in archaea, suggesting a truly ancient origin [8]. Although its amino acid sequence in the pore region is closer to Ca_Vs, NaChBac selectively conducts Na^+. These properties further confirmed its identity as an ancestor of both mammalian Na_Vs and Ca_Vs. The bacterial Na_Vs are 'minimalist' in structure, as they have a VSM and PM but no large intracellular or extracellular linkers. This minimalist structure makes them ideally suited for structural studies of the conserved core functions of Na_Vs and Ca_Vs. Here we review the mechanistic insights into the conserved core functions of Na_Vs and Ca_Vs gained from structural analyses of the NaChBac family of bacterial ion channels.

The first glimpse of Na_V structure

One decade after identification of NaChBac, the X-ray crystal structure of its ortholog from *Arcobacter butzleri* (Na_VAb) was determined at 2.7-Å resolution [9], opening the way to understanding Na_V structure and function at the atomic level. The Na_VAb structure revealed a homotetrameric architecture in which four subunits pack in a symmetric manner giving rise to a functional channel with a central ion-conducting pore (Figure 2A). The four S5 and S6 helices and the P loops form the pore, which is surrounded by the VSM comprising the S1–S4 helices. Interestingly, although the VSM of each subunit is coupled to the pore via an α-helical S4–S5 linker, its closest noncovalent contacts are with the S5 helix of its neighboring subunit (Figure 2A,B). The S4–S5 linker helix of each subunit also directly intersects the S5 and S6 helices of the adjacent subunit. Such a tightly interlocked subunit arrangement suggests that the four VSMs cooperatively control concerted opening of the pore.

The Na_VAb structure unveiled the detailed anatomy of a Na_V pore. The Na_VAb pore comprises an external vestibule, a narrow ion selectivity filter, a spacious water-filled central cavity, and an activation gate in closed conformation at the inner end of the S6 helices, completely sealing off the ion-conduction pathway (Figure 2C,D). Because Na_VAb activates at very negative membrane potentials,