Science Bulletin 62 (2017) 1291–1292

Contents lists available at ScienceDirect

Science Bulletin

journal homepage: www.elsevier.com/locate/scib

Science Bulletin
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Research Highlight

Uncover the myths of voltage-gated sodium channels: cryo-EM structure of the EeNa$_v$1.4-β1 complex

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Ion channels are membrane-embedded macromolecular pores, which allow charged ions flow through the insulating lipid bilayer when channel pores are opened. The opening and closing (termed gating) of ion channels are modulated by various stimuli, such as changes of membrane potential and binding of ligands (neurotransmitters, toxins, etc.). Some ion channels are classified as "voltage-gated ion channels", as they can be gated by altering the voltage difference across cell membrane. Various voltage-gated ion channels (specific to sodium ions, potassium ions, calcium ions, etc.) function collectively to amplify, transmit and generate electric signals in excitable cells (e.g., nerve and muscle cells), and therefore play a central role in neural signal transduction, muscle contraction and other fundamental physiological processes [1].

Voltage-gated sodium (Na$_v$) channels are critical for generating action potentials. When stimulated by fluctuation of membrane potentials, they undergo three main conformation states (closed, open, and inactivated) to regulate the influx of sodium ions [1,2]. Dysfunctional Na$_v$ channels have been implicated in a series of neurological and cardiovascular disorders. Notably, over 1000 disease-related mutations have been identified in human Na$_v$ channels [3,4]. Moreover, Na$_v$ channels are also major targets for drug development as natural toxins (scorpion venom, snake venom, tetrodotoxin, etc.) and clinical drugs (local anesthetics) act on them directly. However, despite their physiological importance and relevance to numerous diseases, structural elucidation of eukaryotic Na$_v$ channels at atomic or near-atomic level remains a highly challenging task, particularly due to the difficulty to obtain protein samples in sufficient quantity and purity. Recently, an exciting breakthrough has been reported in a study entitled "Structure of the Na$_v$1.4-β1 complex from electric eel", presenting a 4.0 Å cryo-EM structure of a eukaryotic Na$_v$ channel complex in an open state and revealing a potential allosteric blocking mechanism of fast inactivation [3].

In eukaryotes, Na$_v$ channels are comprised of two kinds of subunits: a core α subunit, which is able to conduct sodium ions in a voltage-gated manner by itself; and one or more auxiliary β subunits playing regulatory roles (modulating membrane trafficking, expression and electrophysiological properties of α subunits). The α subunit is folded from a single polypeptide and contains four similar repeats (repeats I-IV), which form a "pseudotetramer". Each repeat consists of six transmembrane segments (TMs) (S1-S6), forming two functional modules: S1-S4 form the voltage-sensing domains (VSDs I-IV). Notably, the highly conserved S4 segments are featured by recurring positively charged lysine and arginine residues. S5 and S6 together enclose the ion-conducting pore domain, responsible for the ion selectivity of Na$_v$ channels. The β subunits generally contain only one N-terminal immunoglobulin (Ig) domain and one TM (Fig. 1). In human, nine subtypes of α subunits (Na$_v$1.1–1.9) and four subtypes of β subunits (β1-4) have been identified, distributing in nerve, muscle, and other excitable tissues [1,3,4].

As the first Na$_v$ channel to be purified and cloned, EeNa$_v$1.4 has been studied extensively and regarded as an important model for investigating the structure and function of Na$_v$ channels. As aforementioned, protein sample preparation was a major hurdle for structural elucidation of Na$_v$ channels. Indeed, the authors had to perform dozens of purification trials before one satisfactory batch of sample could be obtained using a GST-fused antibody fragment (scFv), which recognizes polysialic acid (a large glycan) on EeNa$_v$1.4. Then the authors proceeded to obtain a cryo-EM model of EeNa$_v$1.4-β1 complex with an overall resolution of 4.0 Å. The EeNa$_v$1.4-β1 structure was compared with the Na$_v$PaS structure (from American cockroach), the first eukaryotic Na$_v$ channel structure solved to near-atomic resolution [5]. The structural comparison and additional analyses gave rise to several key discoveries as detailed below.

Firstly, interaction between the auxiliary β1 subunit and α subunit was revealed, leading to a more detailed understanding of the regulatory mechanism of β subunits. Next, the III–IV linker of EeNa$_v$1.4 (situated between VSD$_{III}$ and VSD$_{IV}$, responsible for fast inactivation) is clearly displaced comparing to that of the Na$_v$PaS structure. Particularly, the LFM motif (critical for fast inactivation) is inserted between the inner S4-S5 and outer S6 segments of the pore domain, suggesting an important and novel allosteric blocking mechanism for fast inactivation of Na$_v$ channels. Above all, the most striking discovery is that this EeNa$_v$1.4-β1 structure appears to be locked at an open state. Such a state is extremely difficult to be captured, as it only lasts for milliseconds in the absence of specific ligands or mutations. This makes EeNa$_v$1.4 the first ever eukaryotic Na$_v$ channel whose structure is solved in an open state.

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https://doi.org/10.1016/j.scib.2017.09.022

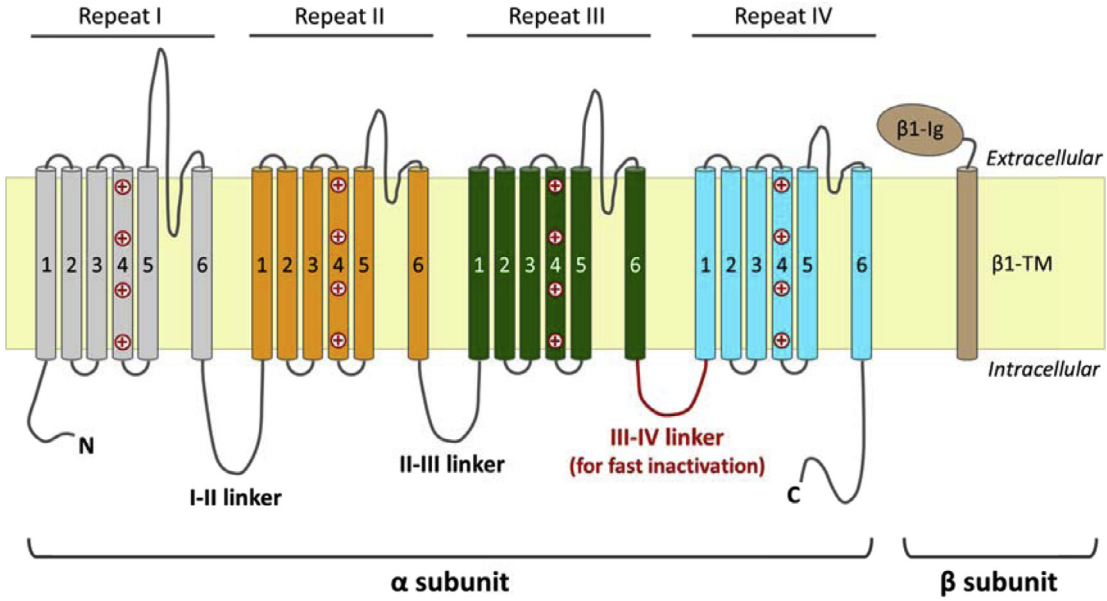

Fig. 1. (Color online) A simplified topology of EeNa$_v$1.4-β1 complex. Cylinders represent the transmembrane segments (TMs), which are numbered respectively. The S4 voltage-sensing segments are annotated with encircled cross marks, representing positively charged residues.

Further analysis of the electron density map revealed a density resembling a detergent molecule (digitonion), which might block the intracellular gate and help stabilizing the channel in an open conformation. Overall, this rare open conformation of EeNa$_v$1.4-β1 has greatly advanced our understanding of the fundamental electromechanical coupling mechanism of Na$_v$ channels and will serve as an important model for structure-based drug design.

Nonetheless, as only part of the EeNa$_v$1.4-β1 domains (pore domain, VSD$_{III}$ and VSD$_{IV}$) are well resolved, a cryo-EM model of atomic resolution is required to fully elucidate the molecular mechanism of this Na$_v$ channel complex. Clearly, the sample preparation is still the key to address this challenge. As the protein sample used for this study actually contains two isoforms of EeNa$_v$1.4 (EeNa$_v$1.4a and EeNa$_v$1.4b), only conserved residues can be assigned reliably. Additionally, β2 and β4 subunits were also identified in the protein sample besides the β1 subunit by MS analysis. Therefore, it is plausible to expect that protein samples of higher purity will lead to cryo-EM models of better resolution, which remains to be addressed and achieved in future studies.

Conflict of interest

The author declares that he has no conflict of interest.

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