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RESEARCH HIGHLIGHT

Calcium channel structures come of age

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A near-atomic resolution structure of a mammalian voltage-gated calcium channel (Ca_v) has been determined. This first fully-assembled Ca_v structure illuminates mechanisms of Ca_v properties and functions and ushers in a new era in Ca_v research and beyond.

Voltage-gated calcium channels (Ca_vs) control movement, heartbeat, hormone secretion and brain activity. Malfunction of Ca_vs due to gene mutations or dysregulation causes cardiovascular, sensory and neurological diseases [1]. Ca_vs are targets of several blockbuster drugs [1]. What do Ca_vs look like? How do the different molecular components of Ca_vs intermingle? What makes a calcium channel a calcium channel? How do Ca_vs gate (i.e., open, close and inactivate)? Where and how do Ca_v drugs bind? How do disease-causing mutations alter Ca_v structure and function? A near-atomic resolution structure of a prototypical mammalian Ca_v obtained by Wu and colleagues and published recently in *Nature* [2] (Figure 1) provides answers or clues to these interesting, important and long-pursued questions.

Ca_vs belong to the superfamily of voltage-gated ion channels. The so-called high-voltage activated Ca_vs are composed of a pore-forming α1-subunit and auxiliary α2δ-, β- and, in the case of the $Ca_v1.1$ complex elucidated by Wu *et al*., γ-subunits (Figure 1A). The α1-subunit, which contains four homologous but nonidentical repeats, dictates the major biophysical and pharmacological properties of Ca_vs, but the auxiliary subunits play key roles in regulating channel gating and trafficking [1, 3, 4].

Crystal structures of Ca_v fragments in complex with calmodulin and of β-subunit in complex with its binding site in the α1-subunit (named the α-interacting domain, or AID) had been obtained some years ago [3, 5] (Figure 1A), and a crystal structure of Ca_vAb, an engineered model bacterial Ca_v formed by four identical single-repeat subunits, has been determined recently [6]. However, before the recent game-changing breakthrough in single-particle cryo-electron microscopy (cryo-EM) [7], high-resolution structures of mammalian Ca_vs seemed unachievable in the next 10 or even 20 years. This is not only because mammalian Ca_vs are composed of multiple subunits, but also because the multi-repeat α1-subunit is large (190-280 kDa) and contains numerous transmembrane helices and several long flexible regions, making them intractable by X-ray crystallography. Moreover, obtaining sufficient amount of pure, homogenous and fully-assembled Ca_v proteins suitable for X-ray crystallography was a daunting challenge.

Wu and colleagues solved the structure of the full $Ca_v1.1$ complex from the rabbit skeletal muscle by using single-particle cryo-EM, which circumvents many of the hurdles confronting X-ray crystallography. The authors used a clever strategy to obtain and purify the native $Ca_v1.1$ complex by replacing the endogenous β1a with a recombinant tagged β1a and taking advantage of the reversibility of the α1/β interaction [3]. A large dataset and sophisticated data processing aided the determination of the final 3.6 Å-resolution structure, improving upon an earlier lower-resolution structure obtained by the same group [8].

The new structure provides unparalleled insights into the mechanisms of Ca_v assembly, ion permeation, gating and regulation. The structure confirms the pentameric architecture of $Ca_v1.1$ (Figure 1B). The four repeats of α1 enclose clockwise to form the asymmetric main body of the channel, with an ion conduction pore in the center. The intracellular end of S6, which forms the inner pore, is tightly closed. This, coupled with the 'up' position of the voltage-sensing S4 helix, suggests that the channel is in an inactivated state. The ion selectivity filter is formed by a ring of carboxylate side chains of the signature 'EEEE locus' and two rings of backbone carbonyl oxygens. This design of employing a combination of negatively charged side chains and main-chain carbonyls to coordinate ions is shared by other calcium-conducting channels, including Ca_vAb [6], TRPV1 [7] and the type I ryanodine receptor [9].

The $Ca_v1.1$ structure reveals how the auxiliary subunits interface with the α1-subunit (Figure 1B). The α2δ-subunit is shown to be cleaved, disulfide-bonded, glycosylated and anchored to the membrane, as previously described [4], with the relevant site(s) identified. α2δ binds several extracellular loops of α1, the amino acid sequences of which diverge among different α1s; thus, the strength of the α1/α2δ interaction likely varies in different Ca_vs. A striking revelation is that α2δ protrudes far into the extracellular space, raising questions of whether it interacts with extracellular matrix proteins and/or other cell surface proteins and how such interac-

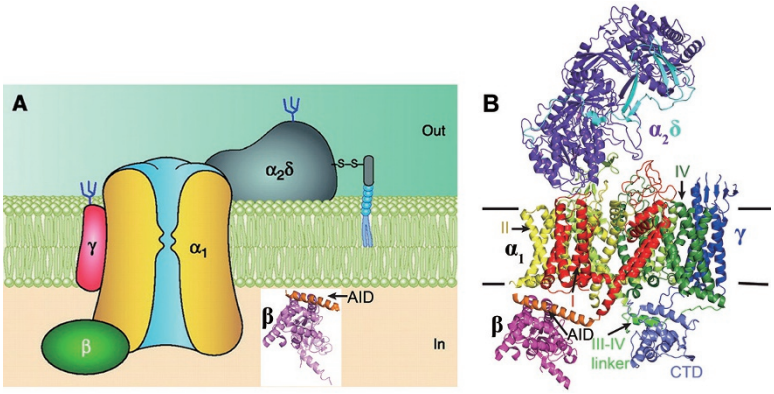

Figure 1 Calcium channel structures — then and now. **(A)** Schematic of a Ca_v complex from a 2010 review [3]. This type of cartoon has been used to depict Ca_vs in the last 30 years. The inset shows a structure of a β-subunit bound with the AID, obtained in 2004 (PDB code: 1VYT). **(B)** Cryo-EM structure of the rabbit skeletal muscle $Ca_v1.1$ complex (PDB code: 5GJV) determined by Wu and colleagues in 2016 [2]. The different subunits are differentially colored, so are the four repeats, the AID, the III-IV linker and the proximal C-terminal domain (CTD) of the α1-subunit.

tions alter Ca_v functions. The β-subunit binds the AID in the same manner as it does in isolated β/AID complexes. However, while the AID was previously envisioned to form a continuous α-helix with S6 of repeat I (IS6) [3, 5], the $Ca_v1.1$ structure shows that the AID helix is discontinuous with IS6 and runs nearly parallel to the membrane. This orientation places some β-subunit regions involved in gating modulation away from the transmembrane domains, suggesting that they may interact with α1 regions unresolved in the structure. The γ-subunit interacts primarily with the voltage-sensing domain of repeat IV, explaining its modulatory effect on gating [3]. Notably, the linker between repeats III and IV (the III-IV linker) of α1 interacts with the proximal C-terminal domain of α1, an interaction that may also modulate gating.

The determination of the first mammalian Ca_v structure marks a watershed in the studies of Ca_v structure-function and Ca_v-centric physiology, pharmacology and channelopathy. It will surely spur structural elucidation of other Ca_vs and investigation of the structural basis of Ca_v drug actions. Among these drugs, dihydropyridines (e.g., amlodipine), phenylalkylamines (e.g., verapamil) and benzothiazepines (e.g., diltiazem) are used to treat hypertension, angina pectoris and cardiac arrhythmias and target the α1-subunit of $Ca_v1.2$, whereas gabapentin and pregabalin are used to treat epilepsy and neuropathic pain and target the α2δ-subunit of Ca_vs in the nervous system [1, 4]. The binding sites of dihydropyridines and phenylalkylamines have been identified recently in Ca_vAb [10], but whether these drugs bind in the same way in $Ca_v1.2$ remains to be determined. Based on the $Ca_v1.1$ structure, more accurate homology structure models of other Ca_vs can now be generated. By the same token, the locations of numerous disease-causing single amino acid missense mutations can now be mapped on the $Ca_v1.1$ structure and their effects on Ca_v structure/function can now be more precisely investigated. With the continuing rapid advance in structure biology, there is good reason to be optimistic that the structures of various macromolecular Ca_v complexes will be solved in the coming years, which will inform the molecular mechanisms of not only Ca_v functions but also excitation-contraction coupling, excitation-transcription coupling and excitation-secretion coupling.

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