Perspective

Functional Subunit Structure of Voltage-Gated Calcium Channels

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VOLTAGE-GATED CALCIUM CHANNELS MEDIATE Ca^{2+} influx into cells in response to depolarization of the plasma membrane. They are responsible for initiation of excitation-contraction and excitation-secretion coupling, and the Ca^{2+} that enters cells through this pathway is important in regulation of protein phosphorylation, gene transcription, and other intracellular events. The activity of voltage-gated Ca^{2+} channels is modulated by a wide variety of cell surface receptors acting through G proteins and protein phosphorylation. The transverse tubule membrane of skeletal muscle has an unusually high density of voltage-gated Ca^{2+} channels. Purification from this source identified α, β, and γ classes of Ca^{2+} channel subunits (*1*), and studies of the biochemical properties and functional reconstitution of purified skeletal muscle Ca^{2+} channels (*2*) have pointed to a complex multisubunit structure for this protein (Fig. 1A) (*3*).

In the skeletal muscle Ca^{2+} channel, a central transmembrane α_1 subunit (175 kD) is thought to be associated with four other polypeptides (*3*). The disulfide-linked α_2 (143 kD) and δ (27 kD) subunits are transmembrane glycoproteins as is the γ (30 kD) subunit, which is noncovalently associated with the other subunits. The hydrophilic β subunit (54 kD) is intracellularly disposed. This subunit structure is surprisingly complex and was initially controversial (*2*). Recent results in this issue of *Science* (*4*), in *Nature* (*5, 6*), and in press in the *Journal of Biological Chemistry* (*7*) more clearly define the structure of this oligomeric complex and suggest essential roles for its constituent subunits in the function of Ca^{2+} channels.

A combination of protein chemistry and complementary DNA cloning experiments have defined the primary structures of all five subunits of the skeletal muscle Ca^{2+} channel (*8*) and the cardiac α_1 subunit (*9*) (Fig. 1B). The α_1, β, and γ subunits are encoded by separate genes. The α_2 and δ subunits are encoded by the same gene and are linked by disulfide bonds and proteolytically cleaved in post-translational processing reactions. The points of interaction among these subunits are not known, but their specific association as a complex is supported by copurification (*1–3*) and by coimmunoprecipitation with antibodies directed against the α_1, α_2, β, and γ subunits (*3, 10*).

Although the Ca^{2+} channel is an oligomeric structure, the α_1 subunit alone is able to function autonomously in some respects. Complementary DNAs or the corresponding messenger RNAs encoding muscle α_1 subunits can direct expression of functional voltage-gated Ca^{2+} channels in recipient *Xenopus* oocytes or mammalian L cells (*9, 11*), and can restore both Ca^{2+} currents and excitation-contraction coupling in myocytes from mice having the *muscular dysgenesis* mutation, which disrupts the endogenous α_1 gene (*12*). What are the roles of the other subunits of the Ca^{2+} channel complex? The new work (*4–7*) employs coexpression methods to provide intriguing, but still incomplete, answers.

Although expression of α_1 subunits in *Xenopus* oocytes or L cells yields voltage-activated Ca^{2+} currents, the amount of current and the number of high-affinity binding sites for dihydropyridine Ca^{2+} channel blockers are very low, and the time course and voltage dependence of Ca^{2+} channel gating differ sharply from those of the native channels. Coexpression of α_2/δ, β, and γ subunits corrects many of the functional defects of the α_1 subunits expressed alone and increases the amount of Ca^{2+} current dramatically for cardiac α_1 expressed in *Xenopus* oocytes. However, the functional effects of the α_2/δ, β, and γ subunits depend on the α_1 subtype and the recipient cell under investigation.

Skeletal muscle α_2/δ subunits substantially increase the amount of Ca^{2+} current in *Xenopus* oocytes expressing cardiac α_1 subunits (*4, 9*). Coexpression of α_2/δ also accelerates activation of the channel significantly and both accelerates and shifts the voltage dependence of inactivation toward more negative membrane potentials (*4*). The combination of α_2/δ and β, but neither subunit alone, shifts the voltage dependence of Ca^{2+} channel activation to more negative membrane potentials (*4*). In contrast to these dramatic effects of α_2/δ on cardiac α_1 expressed in oocytes, relatively little effect of α_2/δ coexpression is observed on skeletal muscle α_1 subunits expressed in L cells (*5*).

Skeletal muscle β subunits have dramatic effects on the functional expression of both skeletal muscle and cardiac α_1 subunits (*4–7*). Coexpression of β subunits in L cells accelerates activation and inactivation of skeletal muscle α_1 subunits more than tenfold (*5, 6*). Coexpression with cardiac α_1 subunits in *Xenopus* oocytes greatly increases the Ca^{2+} current and accelerates activation and shifts its voltage dependence to slightly more negative membrane potentials (*4, 7*).

Skeletal muscle γ subunits have only modest effects on expression of the skeletal muscle α_1 subunit in L cells (*5*). However, they increase the expression, accelerate inactivation, and substantially shift the voltage dependence of inactivation to more negative membrane potentials for cardiac α_1 in *Xenopus* oocytes (*4*). The γ subunit determines the inactivation properties of the cardiac α_1 subunits expressed in oocytes no matter what other subunit combination is present.

Overall, these results show decisively that efficient expression of Ca^{2+} channels with normal physiological properties is greatly enhanced by coexpression of all five of the subunits previously identified as components of the oligomeric Ca^{2+} channel complex. The α_2/δ, β, and γ subunits can each act in combination with either cardiac or skeletal muscle α_1 subunits to increase expression and to restore some aspect of normal channel function. Evidently, each of these subunits can interact directly with the α_1 subunit as suggested by the subunit structure in Fig. 1A and can modify α_1 expression and functional properties. Moreover, coexpression of the full complement of subunits is required for the cardiac Ca^{2+} channel to generate Ca^{2+} currents with a normal time course and voltage dependence (*4*); this finding implies that the five-subunit oligomer is indeed the physiologically functional Ca^{2+} channel. Thus, the new molecular biological studies nicely complement the previous biochemical work to define the functional subunit structure of the L-type Ca^{2+} channel.

Should the effects of these subunits be considered modulatory, structural, or functional? If the actions of the α_2/δ, β, and γ subunits are primarily to modulate or to provide an appropriate environment for α_1 subunit function, one would expect that their effects might differ for different α_1 subunits and for different cellular environments. On the other hand, if these subunits are direct functional participants in voltage-dependent gating and Ca^{2+} conductance and

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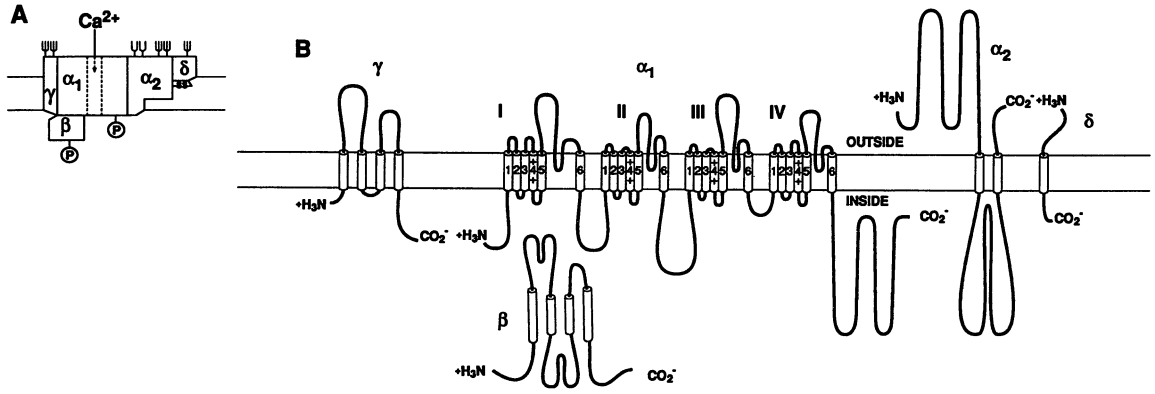

Fig. 1. Subunit structure of skeletal muscle calcium channels. (**A**) A model of the subunit structure of the skeletal muscle Ca^{2+} channel derived from biochemical experiments (*3*). P, sites of cAMP-dependent protein phosphorylation; Ψ, N-linked glycosylation. (**B**) Transmembrane folding models of the Ca^{2+} channel subunits derived from primary structure determination and analysis (*8*). Cylinders represent predicted α-helical segments in the transmembrane regions of the α_1, α_2 δ, and γ subunits and in the peripherally associated β subunit. The transmembrane folding patterns are derived from hydropathy analysis only for α_2 δ and γ and from a combination of hydropathy analysis and analogy with the current models for the structures of Na^+ and K^+ channels (*13*) for α_1. The transmembrane arrangement of α_2 δ is not well defined by hydropathy analysis and the indicated structure should be taken as tentative.

carry out some aspect of these functions themselves, one would expect that the effects of the α_2/δ, β, and γ subunits would be similar or identical when expressed with different α_1 subunits or in different cellular environments. Because each of the subunits has a different functional effect on skeletal muscle α_1 subunits expressed in L cells compared to cardiac α_1 subunits expressed in *Xenopus* oocytes (*4–7*), it seems most likely that these auxiliary subunits are not direct participants in voltage-dependent gating and ion conductance. Instead, they are probably required for the normal function of the α_1 subunit to promote its native structure and to modulate its functions. Since the β subunit is a site of phosphorylation by multiple protein kinases (*2*), its modulation of α_1 subunit function may reflect physiological regulation through phosphorylation pathways. This interpretation of the coexpression results is consistent with current views of the structure and function of the voltage-gated Na^+ and K^+ channels in which voltage-gating, drug-binding, and pore-forming elements are all located in the subunits homologous to α_1 (*13*).

Multiple Ca^{2+} channel subtypes have been described with different physiological and functional properties. At least four physiologically distinct types are expressed in neurons (*14*). Do all Ca^{2+} channel subtypes have a similar functional subunit structure? Several recent results suggest that they may. L-type, dihydropyridine-sensitive Ca^{2+} channels in mammalian brain have α_1-, α_2/δ-, and β-like subunits (*15*). N-type, ω conotoxin–sensitive Ca^{2+} channels in mammalian brain also have α_1, α_2/δ, and β-like subunits (*16*). The expression of a recently cloned dihydropyridine- and ω conotoxin–insensitive Ca^{2+} channel α_1 subunit in *Xenopus* oocytes is dramatically enhanced by coexpression with α_2/δ and β subunits from skeletal muscle (*17*). Although still fragmentary, these results suggest that the complex oligomeric structure of the skeletal muscle L-type Ca^{2+} channel may be recapitulated in most Ca^{2+} channel subtypes. The tissue- and cell-specific functions of Ca^{2+} channels may depend not only on which α_1-like subunit they express, but also on their expression of specific α_2/δ-, β-, and γ-like subunits.

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