

Synaptogenesis: New Roles for an Old Player

The control of synapse formation and shape is important for establishing complex brain circuitry. New evidence now suggests that a component of a synaptic ion channel complex has an unexpected role in these processes.

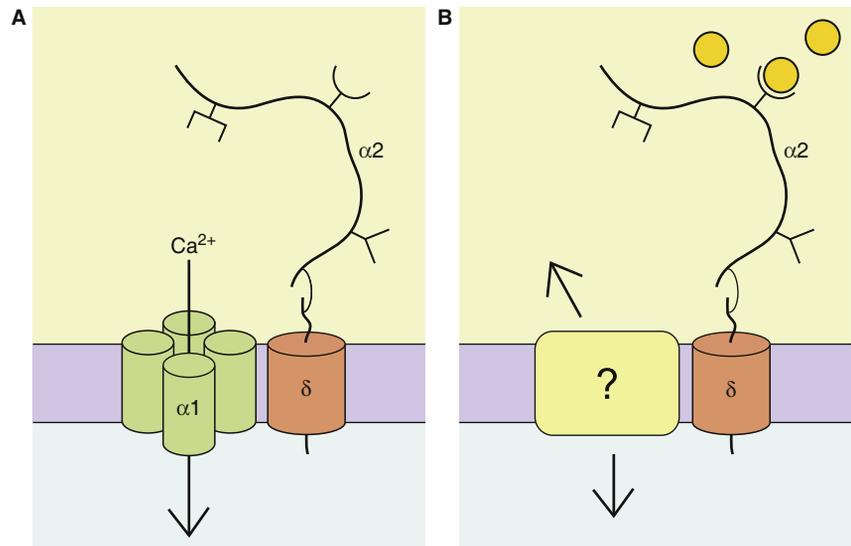
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The synapse is the fundamental unit of connectivity and communication between neurons; trillions exist within the mammalian brain, where they establish the precise circuitry that gives rise to complex thought and movement. It is hypothesized that synapse morphology, strength and synaptic partner choice can all be modulated to promote learning and memory. Numerous protein complexes localize to the synapse and are required for its function. One important complex, the voltage-gated calcium channel, permits calcium entry into the cell following membrane depolarization, allowing for calcium-dependent release of neurotransmitter from presynaptic termini [1,2]. Voltage-gated calcium channels consist of an $\alpha 1$ subunit, which forms the pore through which calcium enters, and several accessory subunits, one of which is the $\alpha 2\delta$ subunit [3]. The $\alpha 2\delta$ subunit associates with $\alpha 1$ and regulates its membrane trafficking, current load and voltage dependence [4,5]. The $\alpha 2\delta$ subunit is translated as a single polypeptide, and then cleaved into two peptides, $\alpha 2$ and δ , which remain linked by disulfide bonds [6]; $\alpha 2$ is entirely extracellular, while δ is predicted to consist of a short transmembrane segment (Figure 1A). A puzzling aspect of $\alpha 2\delta$ biology is its large, highly conserved extracellular domain, which includes protein-protein interaction motifs not required for association with $\alpha 1$ [7]. These motifs suggest that $\alpha 2\delta$ may bind other proteins and have functions beyond its association with $\alpha 1$. Recent work from two research groups [8,9] now suggests that $\alpha 2\delta$ may control synapse formation and morphology, and, importantly, that this role is independent of voltage-gated calcium channel regulation.

In the fruit fly *Drosophila melanogaster*, neuromuscular junction synapses have a characteristic, rounded 'bouton' morphology. Kurshan *et al.* [8] showed that, in animals carrying mutations in the $\alpha 2\delta$ -3 gene, motor axons reach their correct muscle targets, form active zones, and align correctly with postsynaptic receptors; however, boutons never form, suggesting that synaptic morphogenesis does not occur correctly. Bouton formation requires the extracellular $\alpha 2$ peptide but not δ , suggesting that $\alpha 2\delta$ -3 may interact with an extracellular ligand to mediate its effects. Surprisingly, boutons formed normally in animals carrying mutations in the major $\alpha 1$ channel of the *Drosophila* neuromuscular junction. This finding suggests

that $\alpha 2\delta$ -3 regulates synaptic morphology independently of its roles in modulating voltage-gated calcium channels.

How might $\alpha 2\delta$ -3 promote synaptic morphogenesis? A clue may be provided from the recent studies of synaptogenesis in rodents by Eroglu *et al.* [9]. Synapse formation has traditionally been thought to be governed exclusively by the partnering neurons. However, previous studies from the Barres group [10] showed that a second cell type found within the nervous system, glia, may also play a role in synaptogenesis. Usually thought of as passive bystanders with supportive roles, astrocytic glia were shown to induce synapse formation between mammalian neurons in culture. They do this in part by secreting thrombospondins: large, extracellular matrix proteins [11]. In an effort to find the neuronal receptor for thrombospondins, Eroglu *et al.* [9] noted that thrombospondins had been previously shown to bind extracellular protein-protein interaction motifs known as Von Willebrand Factor (VWF) repeats [12]; because $\alpha 2$



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Figure 1. $\alpha 2\delta$ regulates synapse formation and shape by a novel pathway. (A) $\alpha 2\delta$ associates with $\alpha 1$ to regulate the properties of voltage-gated calcium channels. $\alpha 2\delta$ consists of two domains, the extracellular $\alpha 2$ domain and the transmembrane domain δ , which are linked by disulfide bonds. Various protein interaction motifs are found on $\alpha 2$. (B) $\alpha 2\delta$ regulates synapse formation and shape independently of voltage-gated calcium channels: a possible model. Thrombospondins, extracellular circles, bind to the VWF interaction motif of $\alpha 2$, causing a conformational change in $\alpha 2\delta$. This signal is propagated into the cell and perhaps to the extracellular space through another unidentified transmembrane complex.

contains such repeats, they guessed that $\alpha 2\delta$ may be the thrombospondin receptor. This guess paid off handsomely: the authors demonstrated that the $\alpha 2\delta$ -1 protein was required for thrombospondin-induced synaptogenesis in cultured neurons, and that thrombospondins are associated with $\alpha 2\delta$ -1 in brain lysates. The EGF-like domain of thrombospondin was specifically required for synapse induction, and bound to the VWF motif of $\alpha 2$. Furthermore, as in the Kurshan *et al.* [8] study, the synaptogenic properties of $\alpha 2\delta$ -1 were independent of its roles in regulating voltage-gated calcium channels: neither chemical inhibition of $\alpha 1$ channels nor over-expression of $\alpha 1$ affected synapse number.

Together, these studies suggest that $\alpha 2\delta$ may act as a cell surface receptor, regulating synapse formation and shape independently of the $\alpha 1$ channel (Figure 1B). Although it is unknown whether thrombospondins in *Drosophila* bind to $\alpha 2\delta$, evidence from the nematode *Caenorhabditis elegans* suggests that there may be conservation of the ligand across species. Bacaj *et al.* [13] identified a thrombospondin-like protein, FIG-1, which is expressed by glia of sensory organs and regulates properties of their associated sensory neurons. Two $\alpha 2\delta$ -like proteins are found in *C. elegans*, one of which is expressed in the sensory neurons that require FIG-1 [14]. Further studies are needed to determine whether this $\alpha 2\delta$ is indeed a FIG-1/thrombospondin receptor. Interestingly, unlike mammalian thrombospondin-induced synaptogenesis, FIG-1 exerts at least some of its roles independently of the EGF-like domains [13], perhaps indicating that mammalian thrombospondins have other functions yet to be elucidated.

The finding of a new role for $\alpha 2\delta$ proteins is intriguing, and hints at the existence of a novel signaling pathway that regulates synapse formation and morphology. Is $\alpha 2\delta$ required to specify partner identity at the synapse, or does this protein control more global aspects of

synaptogenesis common to many or all synapses? Although this remains an open question, some observations may favor the latter model. First, the $\alpha 2\delta$ thrombospondin receptor localizes to synaptic sites of many neurons [9]. Second, while thrombospondin levels are high in the embryonic mammalian brain at the time most synapses form and are reduced in adults when synapses are generally stable [11], expression is observed throughout the brain. Third, Eroglu *et al.* [9] found that thrombospondin-mediated synaptogenesis via $\alpha 2\delta$ is required for experience-dependent plasticity in the mouse barrel cortex. Perturbation of $\alpha 2\delta$ affected the broad synaptic pattern of the responsive cortical structures in the rodent brain when animals were deprived of sensory inputs by whisker trimming [9]. Fourth, in thrombospondin knockout animals, global changes in synaptic patterning were observed [11]. These observations suggest the tantalizing model that secretion of thrombospondins by glia may define periods when and regions where synaptogenesis can occur.

Kurshan *et al.* [8] suggest that $\alpha 2\delta$ may exert its effects at the synapse through rearrangements of the neuronal cytoskeleton. However, the events that follow engagement of $\alpha 2\delta$ by its ligand are still unclear. Because δ has only a short intracellular domain, which is dispensable for regulating synapse formation and shape [8,9], it is likely that another transmembrane protein associates with $\alpha 2\delta$ and is required to transmit signals into the cell (Figure 1B). It is also unclear whether other $\alpha 2\delta$ -type proteins have similar roles to the ones described in the studies of Eroglu *et al.* [9] and Kurshan *et al.* [8]. If the $\alpha 2\delta$ signaling pathway proves to be conserved across species, as work in *C. elegans* and *Drosophila* may indicate, then these questions might be amenable to study in these genetically tractable organisms. The message for the time being, however, is that $\alpha 2\delta$ is a protein of many hats, reminding us that, sometimes, old proteins can reveal new tricks.

References

1. Robitaille, R., Adler, E.M., and Charlton, M.P. (1990). Strategic location of calcium channels at transmitter release sites of frog

neuromuscular synapses. *Neuron* 5, 773–779.

2. Young, S.M., Jr., and Neher, E. (2009). Synaptotagmin has an essential function in synaptic vesicle positioning for synchronous release in addition to its role as a calcium sensor. *Neuron* 63, 482–496.
3. Arikath, J., and Campbell, K.P. (2003). Auxiliary subunits: essential components of the voltage-gated calcium channel complex. *Curr. Opin. Neurobiol.* 13, 298–307.
4. Gurnett, C.A., De Waard, M., and Campbell, K.P. (1996). Dual function of the voltage-dependent Ca²⁺ channel alpha 2 delta subunit in current stimulation and subunit interaction. *Neuron* 16, 431–440.
5. Felix, R., Gurnett, C.A., De Waard, M., and Campbell, K.P. (1997). Dissection of functional domains of the voltage-dependent Ca²⁺ channel alpha2delta subunit. *J. Neurosci.* 17, 6884–6891.
6. Jay, S.D., Sharp, A.H., Kahl, S.D., Vedvick, T.S., Harpold, M.M., and Campbell, K.P. (1991). Structural characterization of the dihydropyridine-sensitive calcium channel alpha 2-subunit and the associated delta peptides. *J. Biol. Chem.* 266, 3287–3293.
7. Wolf, M., Eberhart, A., Glossmann, H., Striessnig, J., and Grigorieff, N. (2003). Visualization of the domain structure of an L-type Ca²⁺ channel using electron cryo-microscopy. *J. Mol. Biol.* 332, 171–182.
8. Kurshan, P.T., Oztan, A., and Schwarz, T.L. (2009). Presynaptic alpha(2)delta-3 is required for synaptic morphogenesis independent of its Ca(2+)-channel functions. *Nat. Neurosci.* 12, 1415–1423.
9. Eroglu, C., Allen, N.J., Susman, M.W., O'Rourke, N.A., Park, C.Y., Ozkan, E., Chakraborty, C., Mulinyawe, S.B., Annis, D.S., Huberman, A.D., *et al.* (2009). Gabapentin receptor alpha2delta-1 is a neuronal thrombospondin receptor responsible for excitatory CNS synaptogenesis. *Cell* 139, 380–392.
10. Ullian, E.M., Sapperstein, S.K., Christopherson, K.S., and Barres, B.A. (2001). Control of synapse number by glia. *Science* 291, 657–661.
11. Christopherson, K.S., Ullian, E.M., Stokes, C.C., Mallowney, C.E., Hell, J.W., Agah, A., Lawler, J., Moshier, D.F., Bornstein, P., and Barres, B.A. (2005). Thrombospondins are astrocyte-secreted proteins that promote CNS synaptogenesis. *Cell* 120, 421–433.
12. Pluskota, E., Stenina, O.I., Krukovets, I., Szpak, D., Topol, E.J., and Plow, E.F. (2005). Mechanism and effect of thrombospondin-4 polymorphisms on neutrophil function. *Blood* 106, 3970–3978.
13. Bacaj, T., Tevlin, M., Lu, Y., and Shaham, S. (2008). Glia are essential for sensory organ function in *C. elegans*. *Science* 322, 744–747.
14. Frokjaer-Jensen, C., Kindt, K.S., Kerr, R.A., Suzuki, H., Melnik-Martinez, K., Gerstbreih, B., Driscoll, M., and Schafer, W.R. (2006). Effects of voltage-gated calcium channel subunit genes on calcium influx in cultured *C. elegans* mechanosensory neurons. *J. Neurobiol.* 66, 1125–1139.

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