ION CHANNELS

Turning the screw of K^+ channel gating

How does the gate of an ion channel open and close? In the case of voltage-gated channels, this question was laid out in its most elementary form by Hodgkin and Huxley, who predicted that a charged particle within the membrane would sense voltage differences and link them somehow to changes in ionic permeability. Ever since that early insight, we have learned a great deal about the structure of voltage-gated ion channels, particularly about K^+ channels. We know, for instance, that the charged particle is the transmembrane segment S4, and that the gate of the channel is formed by the so-called P-loop, located between segments S5 and S6. The question now revolves around trying to understand how these two regions of the protein interact at the atomic level.

In the September issue of *Neuron*, Gandhi *et al.* addressed this problem using an approach called fluorescence scanning mutagenesis. In this method, a fluorophore is attached to single cysteines introduced in the sequence of the protein. As the fluorescence intensity depends on the environment around the fluorophore, it is possible to measure fluorescence changes (ΔF) in response to membrane depolarization, which correlate with movements of the labelled cysteine.

The authors scanned S4 and measured ΔF during activation of the Shaker K^+ channel. They observed that the residues of S4 could be grouped into three classes depending on the kinetics of ΔF. Whereas some residues tracked the activation of the channel, others tracked both the activation and the slow inactivation that occurs upon prolonged depolarization, and a third group sensed only the slow inactivation. When the residues were mapped onto an α-helix, their distribution resembled the threads of a screw running parallel to each other, an arrangement consistent with the outward helical movement of S4 during activation. In addition, an analogous scan of the pore domain revealed a similar division; some residues responded to both activation and inactivation, whereas others only tracked inactivation. Gandhi *et al.* argue that the changes in fluorescence observed in the pore domain during activation are the result of modifications of the local environment caused by the nearby motion of S4. These findings indicate that gating may involve a direct interaction between the transmembrane helix and a docking site on the pore domain.

Notably, the kinetics of ΔF determined for some of the residues were not symmetrical during depolarization and repolarization, indicating that they encounter a different environment as the S4 segment returns to its original position. The authors interpret this observation as evidence that slow inactivation is accompanied by a global rearrangement of the channel structure that affects the pore domain and most of S4. This rearrangement is likely to stabilize the closed state of the inactivation gate during repolarization and as S4 moves back into the membrane.

The findings of Gandhi *et al.* together with a recent report on the involvement of the amino-terminal T1 domain in channel gating are beginning to give us insight at the atomic level as to how channel proteins open and close. A future challenge for the field will be to establish the relationship between all of the regions of the channel involved in the gating process.

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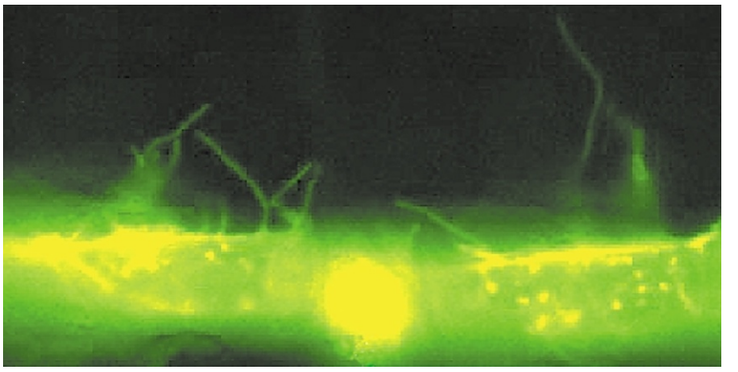

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SYNAPTOGENESIS

It takes two to tango

The current view of synaptogenesis is of a one-sided affair with the axon leading the dance that eventually leads to synapse formation. Indeed, there is a large body of data describing the intricate and dynamic extension of motile filopodia that actively search the environment for suitable partners with which to form appropriate synapses. The role of the axon in actively seeking various molecular cues that facilitate axon guidance is a well established phenomenon that has been extensively studied and is, as a result, a relatively well understood process. In contrast, apart from the provision of the guidance cues, the role of the target cell is less well understood and it is, almost by default, characterized as a passive rather than a dynamic partner in synaptogenesis. Part of the problem is the very nature of the dendritic architecture, which presents substantial technological difficulties to the scientist wishing to study postsynaptic events in synaptogenesis.

As so often seems the case, the more tractable neuromuscular system now provides evidence of a more dynamic role for the postsynaptic tissue in synapse formation. In the October issue of *Nature Neuroscience*, Ritzenthaler, Suzuki and Chiba report that postsynaptic filopodia in muscle cells interact with innervating motor neurons and may force a reconsideration of the dynamics of pre- and postsynaptic events that lead to synaptogenesis.

Ritzenthaler and colleagues studied motor neuron targeting in *Drosophila melanogaster* embryos using high resolution *in vivo* time-lapse imaging to study the formation and properties of the postsynaptic microprocesses (myopodia) that are formed by the embryonic muscle tissue during neuromuscular synaptogenesis. Electron microscopy was used to study the morphology of muscle cells during the period when motor neuron axons start to make contact with the muscle tissue and form synapses. These experiments revealed that embryonic muscle cells extend numerous myopodia just before synaptogenesis and that these processes possess actin-based structural elements. Moreover, myopodia formed clusters at sites of motor neuron innervation and intermingled with innervating axon filopodia. Finally, the authors showed that the clustering was not observed in *prospero* mutants, which have severe delays in axon outgrowth, indicating that the clustering depends on axon innervation. These elegant studies suggest that the postsynaptic myopodia are dynamic rather than passive partners in the dance of synaptogenesis.

Peter Collins

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