A loss, or two gains?

Complex nervous system patterning—usually assumed to have coevolved with advanced, centralized nervous systems—may have arisen before nerves consolidated into a central nerve chord, according to Christopher Lowe, John Gerhart (University of California, Berkeley, CA), Marc Kirschner (Harvard Medical School, Boston, MA), and colleagues.

Their idea runs counter to the prevailing theory of dorsoventral axis inversion. The ventral nerve chords in arthropods (such as *Drosophila*) and dorsal nerve chords in chordates (such as humans) have been thought to be related via an inversion event some time during evolution. In the new theory, however, the original ancestor is proposed to have had a dispersed nervous system that converged centrally in independent dorsal and ventral events.

Reconstructing chordate evolution is tricky for several reasons. The rapidity of the Cambrian explosion and the soft bodies of the ancestors of chordates make it impossible to construct precise evolutionary trees. And chordates’ closest major relatives, the echinoderms, have added so many bizarre anatomical features that they are next to useless for comparisons. Thus, the new study subject is the acorn worm. These hemichordates are a lesser-known lineage but, like the chordates, they are bilateral and their nervous systems coevolved with advanced, centralized nervous systems—usually assumed to have formed in two different ways. The evidence here is split: the chords from arthropods and chordates are started off by two similar inducing molecules but finished off by very different molecules.

Lowe acknowledges that during evolution acorn worms may have lost a central nervous system rather than having failed to gain it. He expects that more examples like the acorn worm in other branches of the evolutionary tree will help support the new model. But, he warns, “this is going to continue to be a really difficult problem to resolve.”


Nervous picket fences

A neuron is a dual-function device—it does both input and output—but it has only one continuous plasma membrane. Protein transport helps to define the two different compartments, but without a barrier membrane, proteins will eventually intermix. Now, Chieko Nakada, Akihiro Kusumi (ERATO and Nagoya University, Nagoya, Japan), and colleagues confirm that neurons do have a membrane diffusion barrier, and they propose a mechanism by which it is constructed.

A diffusion barrier at the axonal initial segment (IS; the axonal area nearest the cell body) has been proposed before. But there have always been caveats: the introduced dye might have had too far to travel, or the latex bead used during testing might have cross-linked and thus immobilized its target.

The Japanese team used three single-molecule techniques to demonstrate that diffusion of a phospholipid in the IS area decreased more than 800-fold between 6 and 10 d after plating of a neuron. The decrease in other regions of the neuron was only one- to threefold.

The diffusion barrier arose coincident with the concentration of actin, ankyrin, and various transmembrane proteins in the IS area, and partial disruption of actin made the lipid mobile once again. This reminded Kusumi of his earlier results in nonneuronal cells, in which lipids appeared to “hop” between actin-dependent plasma membrane compartments.

The team thus adopted a version of this model in which actin cables provide the scaffolding, and “transmembrane proteins act like pickets for the fence,” says Kusumi. But could such a scheme explain not just slowed diffusion but a definitive barrier? The team modeled the behavior of membrane proteins and lipids and found that, sure enough, the diffusion rate decreased by a factor of 500 when the boundary coverage by the transmembrane “pickets” increased from 5% to just 25%. Thus, says Kusumi, “you don’t have to close off the boundary completely. Lipids next to immobile proteins become harder to move . . . so the barrier function propagates.”

**Sticky transmission**

Nerve cells restrict neurotransmitter release to their synapses. That restriction, say Markus Missler (Georg-August Universität, Göttingen, Germany), Thomas Sudhof (University of Texas Southwestern, Dallas, TX), and colleagues, is provided in part by the synaptic proteins α-neurexins. These cell adhesion proteins promote calcium channel activity, and thus fusion of neurotransmitter vesicles, at the synaptic junctions.

The polymorphism and adhesion properties of neurexins led researchers to suspect a synapse-forming function. But Missler and coworkers found that mice lacking all three synapse-forming function. But Missler and coworkers found that mice lacking all three α-neurexins had ultrastructurally normal synapses. The mice breathed with difficulty and died on the first day after birth. In the brain stem, where breathing rhythms are generated, synapses showed reduced frequency of spontaneous transmission—a possible sign of presynaptic problems. Reduced amplitudes of synaptic responses were also evident after stimulation of neurons in the neocortex.

The reduced transmission was not further reduced by drugs blocking N-type calcium channels, suggesting that neurexins normally help the channels to function. Channels were still made and transported to the cell membrane. Thus, channels may no longer be localized to the synapse or, as Missler suggests, no longer get activated. Consistent with the latter idea, whole cell calcium currents from the cell bodies were also reduced in mutants.

According to the activation hypothesis, “in neurons, unlike in other tissues, a negative clamp may exist on the function of calcium channels, and neurexin is needed to remove this clamp,” says Missler. “It may sound a bit out of the blue. But neurons have to control the number of active calcium channels very tightly because of the negative consequences of excessive calcium influx. So neurexin may provide a localized activation of calcium channels at synapses.”


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**Activation by reduction**

A plant signaling protein is turned on when its intermolecular disulfide bonds are reduced and it splits into a monomeric form, say Zhonglin Mou, Weihua Fan, and Xinnian Dong (Duke University, Durham, NC). Only the monomeric form of this NPR1 protein can enter the nucleus and activate transcription.

NPR1 is made constitutively, but activated by salicylic acid (SA) as part of a general plant defense against infection. Dong initially set out to purify the NPR1 complex by gel filtration. She was puzzled that an NPR1 peak was found only with SA-treated samples, but then discovered that in the uninduced samples DTT mimicked SA: it liberated monomeric NPR1 from a complex that was too big to enter the column.

SA is produced when plants first blast away at infecting microbes with oxidants, with SA prompting various enzymes to boost oxidant production. The cells then overcompensate with antioxidants.

It is this later reducing environment that unhinges NPR1 from an oligomeric complex, say the researchers. Cysteine-substituted NPR1 mutants were constitutively monomeric and constitutively active in inducing downstream PR genes, which provide longer-term antimicrobial defenses.

The intermolecularly bonded storage form appears to be novel for signaling networks, although intramolecular disulfide formation is known to drive conformational changes that either activate transcription (by bacterial OxyR) or conceal a nuclear export signal (in yeast yAP1).


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**Doing the CDC20 shuffle**

For perhaps a decade, Suc1 was the hardly little protein that featured in almost every cell cycle paper. But it was used only as a reagent—its binding to cyclin-dependent kinases (such as Cdc2 or Cdc28) yielded nearly pure MPF.

Then the budding yeast version, called Cks1, was found to be essential for growth. Now, May Morris, Steven Reed, and colleagues (The Scripps Research Institute, La Jolla, CA) have found that Cks1 helps shuffle proteins, including Cdc28, at the critical CDC20 cell cycle promoter, thus helping push cells through mitosis.

Cdc20 activates the ubiquitination machinery, which then destroys mitotic cyclins and sister chromatid glue to initiate anaphase. Morris stumbled on CDC20 because its overexpression suppressed a cks1 mutant. She then found that Cks1 helped turn on CDC20 expression during mitosis, and that Cks1 and Cdc28 both localized to the CDC20 promoter. But a version of Cdc28 that interacts poorly with Cks1 was actually stuck to the promoter more avidly, suggesting that Cdc28 binds the promoter first, then brings in Cks1, and finally is kicked off via a Cks1-related mechanism.

Indeed, at the peak of both CDC20 expression and Cks1 binding to the promoter, Cdc28 was released. The release may be triggered by the proteasome, which cofractionates with Cks1 and can also bind transiently to the CDC20 promoter. Proteasome protease function is not necessary for CDC20 expression, so perhaps the proteasome is recycling a promoter-bound protein, such as Cdc28, after it has fulfilled some essential phosphorylation function, or remodeling either chromatin or a stalled transcription complex.