Research Roundup

Sights on cone geometry

Rather than merely point-to-point dispatches, synaptic messengers also make widespread broadcasts, as evidenced by Steven DeVries, Wei Li, and Shannon Saszik (Northwestern University, Chicago, IL). Messages are received both near and far, they show, to create transient and sustained responses.

The cone presynaptic terminal is highly invaginated, with ribbons of glutamate-containing vesicles above each invagination. Cones respond to changes in light with graded changes in membrane potential. Decreases in light intensity depolarize cones and increase glutamate release, which then activates a class of cells known as Off bipolar cells.

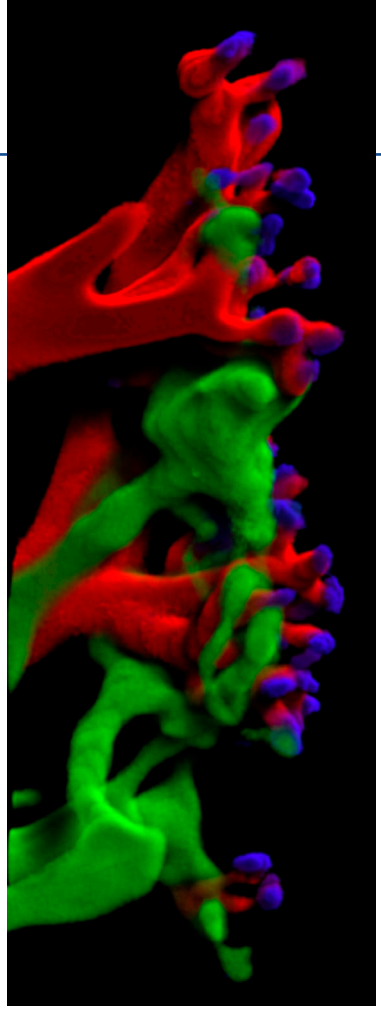
In the new report, DeVries et al. show that Off bipolar cell dendrites contact cone terminals at two sites. Most subtypes of Off bipolar cells contact the base of the cone terminal, ~300 nm away from the vesicle fusion sites. The group found, however, that one Off cell subtype extended its dendrites up into each invagination to end close to fusion sites.

These contacts within invaginations experienced large, rapid fluctuations in glutamate levels when a cone was depolarized. Glutamate then spilled out of the invaginations to the basal contacts. In spite of their distance from release sites, even a single vesicle's worth of glutamate was able to reach and activate these cells. Distance exacted a toll, however, as the glutamate concentrations sensed by these cells fluctuated more slowly and at much lower levels.

The invaginating cell senses glutamate via AMPA receptors, which recover rapidly from glutamate-induced desensitization and can thus decode rapid consecutive pulses. Basal cells instead use kainate receptors, which have much slower recovery times and produce responses that average over rapid fluctuations in glutamate concentration. The basally located Off bipolar cells thus generate more sustained responses.

The steady signal conveys the basic sight information of change magnitude and duration. The transient signal saying just that there was a change "is probably very important," says DeVries, "because it can help an animal avoid predators or moving objects." JCB

Reference: DeVries, S.H., et al. 2006. *Neuron.* 50:735–748.



DEVRIES/ELSEVIER

AMPA receptors (blue) of one Off bipolar cell subtype (red) extend into cone invaginations, unlike the dendrites of another subtype (green).

Alien sensing by GC content

A bacterial protein silences foreign DNA by recognizing low GC content, as shown by William Navarre, Ferric Fang (University of Washington, Seattle, WA), and colleagues. The silencing might allow the cell to experiment evolutionarily with intruding DNA.

The silencing protein is a *Salmonella* histone-like protein called H-NS. The group was searching for direct targets of this known repressor when they noticed that the H-NS binding sites were GC poor (~47%) compared with the rest of the chromosome (~52%). A GC-poor foreign gene that the group recombined into *Salmonella* was also repressed by H-NS.

Most bacteriophage and other bacteria are lower in GC content than *Salmonella* and its relatives, so invading DNA is an obvious target for H-NS. "It's like a primitive immune system," says Fang. "Reduce their expression, and the foreign genes can be tolerated."

Useful newcomers might eventually be expressed, however, via mutations that increase their GC content or through the evolution of antisilencers. Many of *Salmonella's* foreigner-derived virulence genes, for instance, are shut off by H-NS but can be reactivated when needed by a transcription factor called SlyA.

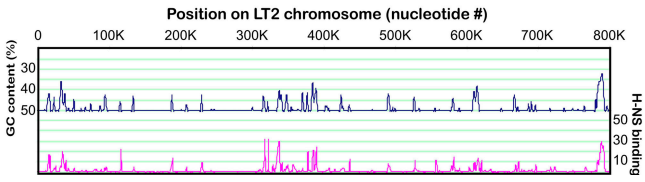
Bacteriophage, of course, also evolved means to get around this defense system. Some encode their own H-NS antagonists, whereas others maintain GC-neutral genomes.

Other GC-rich bacteria have related DNA-binding proteins that are possible analogues of H-NS. Bugs with AT-rich genomes might in turn have GC-binding repressors. If universal, this immune strategy would explain why each bacterial species maintains its distinctive GC/AT ratio.

H-NS seems to recognize short stretches of DNA, although how the protein reads GC content within just a couple hundred base pairs is not clear. Perhaps it recognizes the intrinsically curved or partially melted structure of AT-rich sequences.

Others have found that tandemly bound copies of H-NS form multimers. This probably blocks transcription by compacting the DNA in that vicinity. By locking many helical turns in place, it would also restrict changes in DNA superhelicity, thus explaining the known repressive effect H-NS has on heat- and salt-induced responses. JCB

Reference: Navarre, W.W., et al. 2006. *Science.* doi:10.1126/science.1128794.



FANG/AAAS

Chromosomal sites of low GC content (blue spikes) coincide with H-NS binding (pink spikes).

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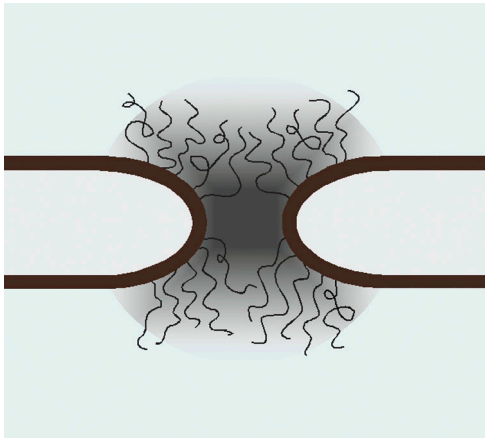

LIM

Flexible FG Nups (black) create an entropic barrier (gray) to the nuclear pore.

Entropy guards pore

The rapid fluctuations of flexible FG repeat–containing nucleoporins (FG Nups) form an entropic barrier to would-be entrants into the nuclear pore complex (NPC), according to Roderick Lim (University of Basel, Switzerland) and colleagues.

FG Nups, which consist of large natively unfolded domains, are the pore's gatekeepers—they keep out proteins that are not bound to transport receptors. Thus, says Lim, "the mechanics of transport lies in how FG domains behave at the nanoscale."

To examine this behavior, the group used atomic force microscopy on clusters of one such FG domain, called cNup153, tethered at one end to gold nanodots. The forces exerted by the cluster just nanometers above the dot were reminiscent of the behavior of a physical phenomenon known as polymer brushes. Specifically, random flexible movements of polymers (or unfolded FG domains) create a large exclusion volume. Interactions between hydrophobic FG repeats were not seen, suggesting that FG Nups do not form a meshwork.

When packed tightly, says Lim, "being entropically dominated means that it is probably unfavorable for the FG domains to remain in confined spaces," such as the center of the NPC. Most FG Nups therefore probably extend out of the pore, creating a corona-like barrier to incoming proteins.

The binding of a transport receptor to an FG domain locks it into an entropically reduced conformation that might thus collapse into the pore. JCB

Reference: Lim, R.Y.H., et al. 2006. *Proc. Natl. Acad. Sci. USA*. doi:10.1073/pnas.0603521103.

Phosphate give and take

A plant hormone receptor has a split personality, revealing its kinase or phosphatase side depending on whether hormone is bound, say Ari Mähönen, Ykä Helariutta (University of Helsinki, Finland), Masayuki Higuchi, Tatsuo Kakimoto (Osaka University, Japan), and colleagues.

Plant hormones known as cytokinins, which stimulate growth and cell division, are detected by three receptors with hormone-induced histidine kinase activity. The loss of cytokinin-binding ability of one of these receptor kinases, CRE1, stunts root growth. This phenotype is suppressed, the group now finds, by mutations elsewhere within CRE1. Based on this result, they hypothesized that hormone-free CRE1 inhibits its immediate downstream elements, which can be phosphorylated by multiple histidine kinases.

A logical countermeasure to a kinase is a phosphatase. Indeed, the group shows, CRE1 is a phosphatase when not bound to cytokinins. Perhaps this bidirectional phosphorelay activity creates more rapid changes in signaling than when kinases and phosphatase are separate. Replacing *Arabidopsis* CRE1 with a kinase-only homologue resulted in cytokinin hypersensitivity.

The cytokinin phosphorelay pathway is analogous to bacterial two-component systems, which are absent from the animal kingdom. Plant light and ethylene receptors are also part of two-component–like networks, although no built-in phosphatase activity is known for these receptors. JCB

Reference: Mähönen, A.P., et al. 2006. *Curr. Biol.* 16:1116–1122.

MÄHÖNEN/ELSEVIER

A CRE1 mutant that cannot bind cytokinins has short roots (left) because it is locked in a phosphatase form.

Axon's vesicles in excess

Excess transport vesicles circulate constantly through the axon and nerve terminal as though on a conveyer belt, based on work from Dinara Shakiryanova, Arvonn Tully, and Edwin Levitan (University of Pittsburgh, Pittsburgh, PA). The constant stream gives active synapses immediate access to a boost in neuropeptides.

Synapses were thought to be at the mercy of the cell soma for the delivery of vesicles containing neuropeptides, which are released upon synapse activation. Levitan and others wondered how this set up allows synapses to be dynamic, since the soma can be far from a nerve terminal. "It can take days," says Levitan, "to get stuff shipped down there, even with fast axonal transport."

The new results "recast the relationship between the terminal and the soma," says Levitan. "The soma sends out excess resources, and terminals decide for themselves how much to use."

Vesicles entered synapses in constant numbers, but after synapse activation their transport out of the synapse was inhibited. Very little is known about regulated retrograde transport, but the authors suggest that the dynein motor might release vesicles—or any cargo, for that matter—upon activity-induced calcium influx.

The excess vesicle flow might seem like a waste of energy, but Levitan compares the scheme to a water plant running water from house to house so everyone gets immediate access. "Each synapse can tap in and get extra vesicles immediately whenever they need it." JCB

Reference: Shakiryanova, D., et al. 2006. *Nat. Neurosci.* doi:10.1038/nn1719.

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