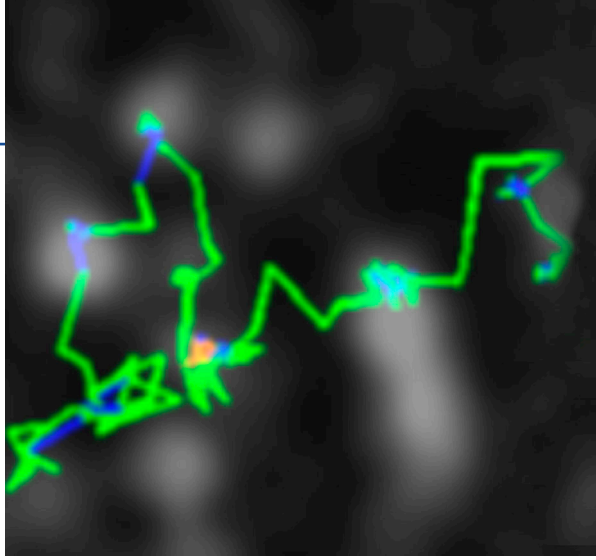
Research Roundup



VALE/ELSEVIER

GFP-tagged LAT molecules become trapped (blue) at CD2 clusters (light gray).

Selective clusters

Adam Douglass and Ronald Vale (University of California, San Francisco, CA) find that interactions between proteins, not lipids, drive the formation of plasma membrane microdomains in signaling T cells.

In active T cells, signal transduction proteins cluster within the plasma membrane, probably to enhance signaling by concentrating the interacting proteins. Popular model mechanisms for clustering involve either lipid rafts or actin. But Douglass and Vale found that the signaling proteins themselves hold clusters together.

In their system, T cell clusters included the LAT adaptor protein, the Lck tyrosine kinase, and the CD2 costimulatory transmembrane protein. LAT and Lck are thought to be raft-localized proteins. But mutation of their raft-localizing regions did not alter LAT or Lck diffusion or clustering. By contrast, mutating LAT residues that are essential for protein–protein interactions prevented LAT clustering.

Clusters were also maintained in the absence of actin polymerization, although actin was needed for cluster formation. Douglass and Vale think that actin or actomyosin may be needed for the initial movement of proteins into clusters, but not for anchoring them together.

When tracking single molecules, the authors noticed that LAT and Lck diffused rapidly outside of clusters but became temporarily trapped, probably via protein–protein interactions, when encountering cluster sites. Nonclustering proteins were rarely trapped and were forced to navigate between clusters.

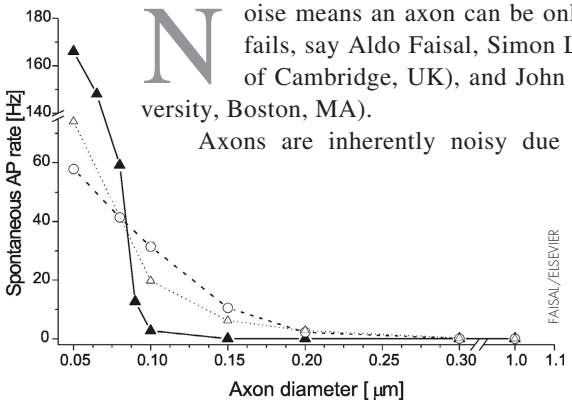
Vale notes that their findings do not rule out the existence of lipid rafts. Rather, the findings support the idea that "protein–protein interactions may be a more common mechanism for creating signaling microdomains," he says. He hopes eventually to understand why microdomains need to be formed during T cell signaling. "Many people in the signaling field think about which molecules interact with one another," he says, "but the issue of how the molecules are organized spatially and how this affects their function is often not addressed." JCB

Reference: Douglass, A.D., and R.D. Vale. 2005. *Cell.* 121:937–950.

Axon size matters

Noise means an axon can be only so small before it fails, say Aldo Faisal, Simon Laughlin (University of Cambridge, UK), and John White (Boston University, Boston, MA).

Axons are inherently noisy due to the spontaneous openings and closings of ion channels that cause membrane potential fluctuations. When the noise becomes too great, a spontaneous action potential ensues, which can disrupt communication between axons. As the rate of this spontaneous firing increases exponentially as axon diameter decreases, Faisal wondered whether channel noise limits axon size.



FAISAL/ELSEVIER

Spontaneous firing increases dramatically below a critical diameter in rat (triangles) and squid (circles) axons.

To test this question, the team developed a mathematical model that tracks axon dynamics when single ion channels "behave badly," or open and close at the maximum threshold observed experimentally. Using data from well-studied biological systems, such as specialized cortical rodent and squid axons, they found that axon diameter is the most significant factor affecting spontaneous action potentials; other factors such as channel density, channel conductance, and membrane properties had little effect.

Although the necessary molecular machinery can be packaged into an axon only 0.06 μm in diameter, the model predicted that axon size must be at least 0.10 μm. Below this size, spontaneous axon firing is so prevalent that effective communication between axons becomes garbled. Indeed, the smallest natural axons that they found were 0.10 μm in diameter, with a few unusual exceptions.

The mechanism driving action potentials is one of the best-studied cellular signaling systems, but "it is not well-appreciated that these biological systems are not perfectly reliable," says Faisal. Recognizing that noise is inherent in biological signaling systems at the nanometer scale is important both for studying cells and for applying nanotechnology founded on similar biomolecular mechanisms. JCB

Reference: Faisal, A.A., et al. 2005. *Curr. Biol.* 15:1143–1149.

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Sensing voltage for function

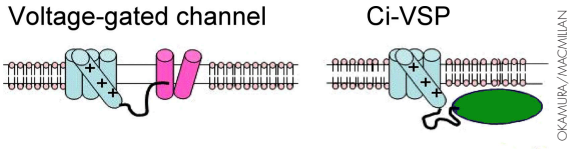
The first protein to sense changes in membrane potential but not function as an ion transporter is now identified by Yoshimichi Murata, Hirohide Iwasaki, Mari Sasaki, Yasushi Okamura, and colleagues (National Institutes of Natural Sciences, Aichi, Japan). Activation of this sensor probably induces phosphorylation-based signaling events.

The group discovered this sea squirt protein, Ci-VSP, based on its sequence homology with ion channels. But the homology was confined to four transmembrane segments that comprise a voltage sensor. This domain functions just like the voltage sensor domains of channel proteins. Ci-VSP also contains a cytoplasmic phosphatase domain, but lacks a pore domain to transport ions across the plasma membrane.

Using an in vivo bioassay, Okamura's group showed that Ci-VSP changes cellular phosphoinositide concentrations in response to membrane potential changes. "These data provide the first evidence since the Hodgkin-Huxley age that a molecular function other than that of ion channels is regulated by membrane voltage," he says.

Ci-VSP is expressed in sperm and might function in sperm motility or morphology. The authors' next goal is to identify the natural substrate of Ci-VSP, which they suspect is PIP_3, so that they can determine whether membrane hyperpolarization or depolarization activates the enzyme. JCB

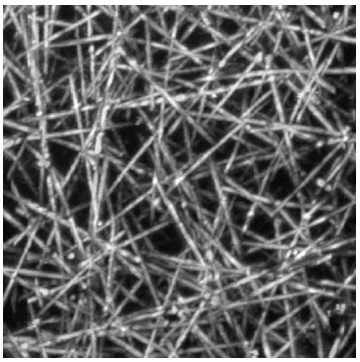
Reference: Murata, Y., et al. 2005. *Nature.* 435:1239–1243.



Ci-VSP's voltage sensor (blue) regulates a phosphatase domain (green), not a pore domain (pink), as in voltage-gated channels.

Fiber flex

Fibrin fibers bend much more than they stretch, according to Jean-Philippe Collet, John Weisel (University of Pennsylvania, Philadelphia, PA), and colleagues. This flexibility lends the necessary elasticity to blood clots.



A blood clot is composed of ligated fibrin fibers that bend more than they stretch.

Blood clots, which are composed of fibrin fibers, are both elastic and plastic—they mostly return to their original form after stretching but can also be irreversibly deformed. This viscoelasticity makes clots stiff enough to stem blood flow but pliable enough not to become obstructive.

Weisel's group investigated the mechanical properties of individual fibers that confer viscoelasticity to clots. They used laser tweezers to pull on beads attached to fibrin fibers within clots that were prepared from blood plasma. By measuring the force required to displace the bead a given distance, they calculated the fiber stiffness and found that individual fibrin fibers are 300 times more pliant for bending than they are for stretching. "From these measurements and from the clot structure," says Weisel, "we can say that fibrin is not rubber-like," which some scientists had previously hypothesized to account for clot elasticity.

Fibers could be stiffened nearly tenfold by the addition of factor XIIIa, an enzyme that stabilizes clots by creating covalent linkages between fiber molecules. Weisel's group now plans to model how the mechanical properties of individual fibers relate to the viscoelasticity of whole clots. Weisel notes, "I hope this research gets the attention of clinicians as well as researchers, because the mechanical properties of clots are important for understanding their function and pathology." JCB

Reference: Collet, J.P., et al. 2005. *Proc. Natl. Acad. Sci. USA.* 102:9133–9137.

Smell's different

G protein amplification occurs automatically in an activated rod cell, but not so simply in olfactory cells, according to Vikas Bhandawat, Johannes Reisert, and King-Wai Yau (Johns Hopkins University, Baltimore, MD).

Photon activation of a single rhodopsin molecule activates many G proteins, thereby amplifying the signal until rhodopsin is inactivated by phosphorylation. "Based on this one well-studied system, it has generally been assumed that other G protein pathways behave similarly," says Yau. Now, his group's analyses of single olfactory receptor neurons reveal a low amplification system.

An individual odorant-bound receptor exhibited a very low probability of activating even one downstream G protein molecule, as odorant receptor binding was transient—lasting 1 ms or less. "We expect many other ligand-triggered G protein pathways to behave similarly," says Yau.

Olfaction amplification therefore requires increasing the probability of G protein activation. This could be achieved either via many odorant molecules that continuously bind to receptors, or via a large number of receptors, so that odorants at low concentrations will still be able to find a receptor. "When these events are summated across all receptor molecules on the cell, and all cells express the same receptor protein," says Bhandawat, "this produces substantial signal amplification and therefore high sensitivity in the brain." JCB

Reference: Bhandawat, V., et al. 2005. *Science.* 308:1931–1934.