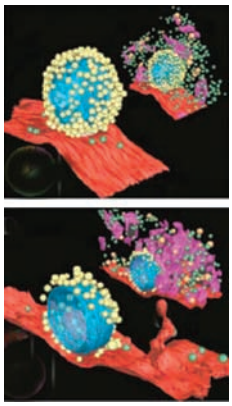
Research Roundup

Vesicles slide on a synaptic body

In vision and hearing, all-or-nothing action potentials are insufficient because they cannot transmit small changes in intensity. Thus, neurons in these areas use the synaptic body (SB), an unusual presynaptic organelle that is specialized for continuous transmitter release. This requires a lasting supply of synaptic vesicles. A new article by David Lenzi, William Roberts (University of Oregon, Eugene, Oregon), and colleagues suggests that the SB is a source of these vesicles.



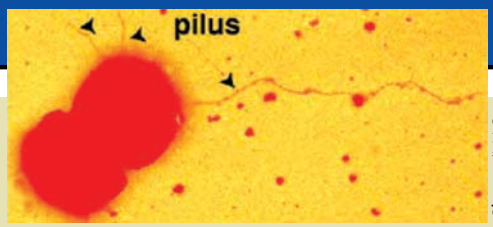
Roberts/Elsevier

A gradient of synaptic vesicles (yellow) on the SB (blue) remains after excitation (bottom).

The distribution of vesicles on the SB changes sharply in response to stimulation, as seen in electron tomography maps of inhibited versus strongly stimulated synapses. Vesicles near the plasma membrane side of the SB were released most rapidly. This generated a gradient of vesicles, most of which remained on the cytoplasmic face of the SB. "The redistribution is what you expect if vesicles bind to the synaptic body and then move along to reach the release site," says Roberts.

After stimulation, fewer vesicles were found at the plasma membrane just below the SB than in a surrounding ring. Thus, stimulation establishes vesicle gradients on both the SB and the plasma membrane that may be sources for ongoing release. Roberts hopes to determine whether the movement of vesicles down the SB is passive or is powered by a transporter. So far, only three SB components are known, which limits the number of proteins that could be tested for transporter activity. ■

Reference: Lenzi, D., et al. 2002. *Neuron.* 36:649–659.



Sheetz/NAS

The destruction of one pilus by PilT releases enough energy to rein in the bacterium.

PilT: a motor on steroids

Many gram-negative bacteria, including the human pathogen that causes gonorrhea, use type IV pili for attachment to host cells. Retraction of the pilus is strong enough to propel a bacterium along cells. Berenike Maier, Michael Sheetz (Columbia University, New York, NY), and colleagues have now measured just how much force is released by pilus retraction, a process known to require the PilT ATPase. Their findings make PilT the strongest known molecular motor.

The strength of pilus retraction was measured by its ability to pull an attached bead from a laser trap of varying stiffness. Retraction frequency was proportional to PilT concentration, indicating that PilT is the molecular motor powering retraction. The retraction of one pilus generated forces in excess of 100 pN, between 2 and 20 times that released by kinesin and polymerases.

PilT's exceptional strength may stem from its unusual mode of action. Unlike most motors, which move bidirectionally along filaments, PilT's action was irreversible. PilT apparently powered retraction by dissociating pilus subunits into the bacterial membrane. The dissociated subunits may be unable to reassemble directly into the filament. PilT belongs to an ATPase family that works in hexameric form and may therefore hydrolyze up to six ATP molecules for each pilus subunit released. This would generate several times more energy than is required for disassembly. As yet, no analogous motors have been found in eukaryotes. If they are prokaryote-specific, PilT-like motors may be convenient targets for drugs to treat certain bacterial infections. ■

Reference: Maier, B., et al. 2002. *Proc. Natl. Acad. Sci. USA.* 99:16012–16017.

Neurons go toward the light

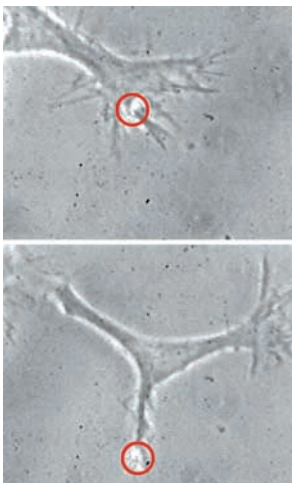
Like a miniature version of the famous tractor beams of sci-fi lore, beams of light known as optical tweezers have been used to hold and manipulate anything from atoms to cells. Now, Allen Ehrlicher, Josef Käs (Universität Leipzig, Germany), and colleagues show that laser beams can also manipulate a biological process—the growth of an axon.

In their new report, the authors direct axonal growth by focusing a beam of light just ahead of the leading edge of a lamellipodium. Forces of light lower than those used for optical tweezers accelerated growth at the leading edge. Shining the laser on one side of the growth cone caused filopodia to accumulate on that side, thereby initiating as much as a 90° turn of the growth cones.

Although the mechanism behind the effect of light is yet unproven, Ehrlicher describes the group's favorite explanation as "biased monomer diffusion." According to the theory, the electrical field gradient created by the beam, which polarizes the nearby pool of actin monomers, draws the monomers toward the beam's focus, thus favoring polymerization. "In effect, we trick the actin polymerization process," says Ehrlicher.

Whatever the mechanism, Ehrlicher is excited by the possibilities that light control over cell motility may offer. One immediate use will be to construct more precise and longer-lasting in vitro neural networks, in which a small number of neurons are connected in a specific fashion to study neuronal interactions. A long-term goal is to use lasers in vivo to guide the regrowth of severed peripheral nerves in accident victims. ■

Reference: Ehrlicher, A., et al. 2002. *Proc. Natl. Acad. Sci. USA.* 99:16024–16028.



Käs/NAS

A growth cone extends as directed by a beam of light (circle, top to bottom).

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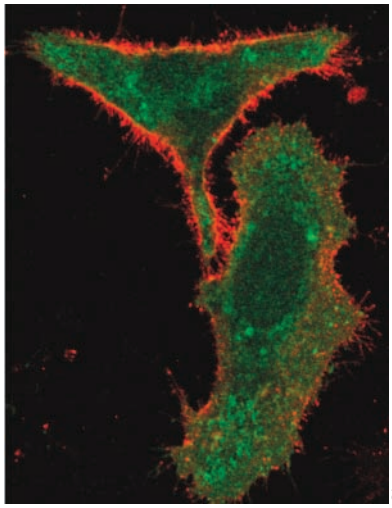
The brightest dots in the bunch

Quantum dots (QDs), fluorescent inorganic crystals <10 nm in diameter, offer several advantages over organic dyes for labeling biological samples. They resist photobleaching, and their emission spectra are narrow enough that multiple variants (excited by one wavelength) can be distinguished in a given sample. The feasibility of using QDs for biological uses is proven in two recent articles by Jyoti Jaiswal, Sanford Simon (Rockefeller University, New York, NY), and colleagues and by Xingyong Wu, Marcel Bruchez (Quantum Dot Corporation [QDC], Hayward, CA), and colleagues. "Quantum dots were offered up as a savior for protein labeling a couple of years ago," says Simon. "But it hadn't been shown that they could be used in vivo."

The QDC group shows that QDs can be used to label proteins in fixed and living cells. Using two colors of IgG- and streptavidin-attached QDs, they simultaneously identified both nuclear and plasma membrane proteins with only a single filter set in fixed cells. QD emissions were four to nine times brighter than Alexa, one of the brightest organic dyes.


Simon

QDs light up membrane-bound Pgp (red), a multidrug transporter, in living cells.

Simon's group extended the usage to long-term labeling of living cells. QDs covered with a stable chemical coat were taken up by endocytosis or were linked to antibodies to label specific membrane proteins. As the label did not affect cell growth or development, the stability of QD fluorescence allowed the authors to follow developing amoebae over several hours and mammalian cells for over 12 d. Simon looks forward to using QD technology to examine protein dynamics during vesicle fusion to the plasma membrane, a process not well suited to the rapidly photobleached GFP.

In the future, nonendocytic organelles may be specifically labeled by conjugating QDs to a signal peptide that will target them to the desired organelle. Labeling of specific nonmembrane-associated proteins inside a living cell may be done by conjugation of the QDs to protein-specific ligands or antibodies. However, for now, those labels will have to be delivered into the cell using the same invasive techniques as for conventional membrane-impermeant dyes. ■

References: Jaiswal, J., et al. 2002. *Nat. Biotech.* 10.1038/nbt767.
Wu, X., et al. 2002. *Nat. Biotech.* 10.1038/nbt764.

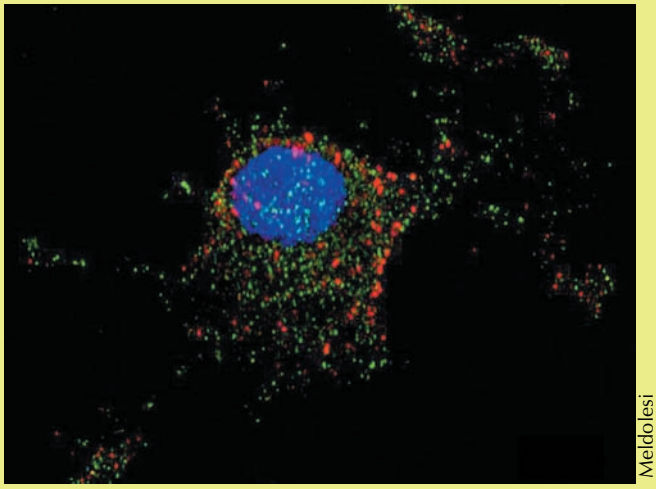
Vesicles that promise enlargement

Regulated exocytosis has its own organelle, according to Barbara Borgonovo, Jacopo Meldolesi (Vita-Salute San Raffaele University, Milan, Italy), and colleagues.

Various signals elicit exocytosis. The best-studied example is regulated secretion, in which a specific cargo is delivered outside the cell. But regulated exocytosis can occur in non-secretory cells, begging the question of which organelle in these cells responds to the stimulus. Recently, lysosomes were shown to fuse to the PM in response to damage-induced calcium signals, but they no longer appear to be the only organelle in the arsenal.

By comparing the PM of living cells before and after stimulation, Meldolesi's group has now identified desmoyokin (dA), a marker for regulated exocytosis that appears on the PM in response to increases in intracellular calcium. dA was found in a set of small vesicles within 0.5 μm of the PM that fused rapidly in secretory and nonsecretory cells. The vesicles lacked markers for known organelles, including endosomes, trans-Golgi, and lysosomes, and are thus a distinct class.

The group named the new organelles enlargosomes. "The name we gave them shows what we have in mind [for their function]," says Meldolesi. He thinks the organelles may provide cells with a means to increase PM surface area during wound healing without releasing hydrolytic enzymes. More enlargosomes were found in differentiating cells, which are also expected to be expanding. Enlargosomes may favor a lasting increase in cell size, as they were recycled more slowly than vesicles involved in regulated secretion. ■


Meldolesi

Both enlargosomes (red) and classical secretory vesicles (green) carry out regulated exocytosis.

Reference: Borgonovo, B., et al. 2002. *Nat. Cell Biol.* 4:955–963.