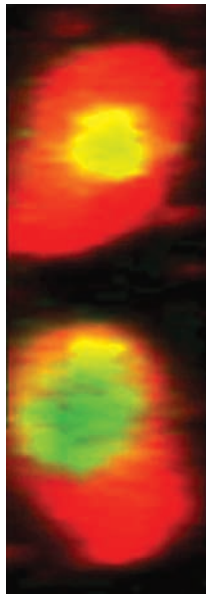


## Connexin the dots

New dyes developed by Guido Gaietta, Roger Tsien, Mark Ellisman (University of California at San Diego, La Jolla, CA), and colleagues allow efficient fluorescent and electron microscopy for studying the location, trafficking, assembly, and turnover of proteins and protein complexes.

“We were interested in being able to go from light microscopy to the EM level,” says Ellisman. “In particular, we wanted better resolution than with immunogold labeling.” To do so, the group examined FAsH, a biarsenical



New connexin (red) is added to the outside of existing plaques (green).

Ellisman/AAAS

derivative of fluorescein. FAsH binds to small amino acid extensions containing the sequence C-C-X-X-C-C, which can be added to recombinant target proteins. Binding of FAsH causes the ligand to emit a strong green fluorescence, and binding of ReAsH, a red variant, leads to red fluorescence.

ReAsH is the variant that is useful for EM, because it can generate singlet oxygen upon illumination. Singlet oxygen drives localized polymerization of the substrate diaminobenzidine (DAB) into an insoluble form that can be viewed by EM. “Because the fluorescent label binds directly to the protein you are trying to localize, and the DAB polymer deposits directly nearby the fluorophore, the resolution is better [than immunogold labeling],” says Ellisman. Additionally, this technique does not require the diffusion of large antibodies into the fixed specimens.

The group put their new label to work by examining the protein connexin43,

which multimerizes to form gap junctions. Pulse-chase with first FAsH and then ReAsH revealed newer (i.e., red) molecules of connexin at the outer edge of large clusters of gap junctions known as plaques. Green molecules were concentrated in the center, indicating that older connexins are endocytosed from the middle of the plaques. EM demonstrated that newly synthesized connexins were first sent to nonplaque sites on the cell surface before flowing to the edge of a plaque.

The team expects there will be many uses for the FAsH-ReAsH system, especially in revealing how large complexes, such as receptor patches at synapses, are formed. “Anyone who has a question about how a cell manages the assembly of large macromolecular structures of a protein should benefit from the use of this system,” says Ellisman. ■

Reference: Gaietta, G., et al. 2002. *Science*. 296:503–507.

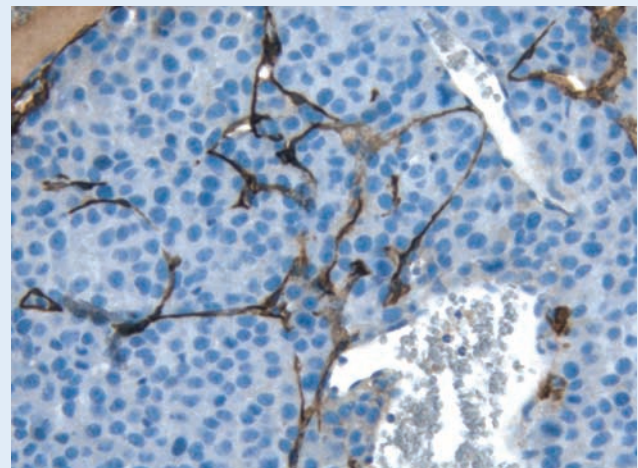
## Widening the target for cancer treatment

Doctors treating cancer patients should aggressively target the area surrounding a tumor during surgery and radiation therapy. That suggestion comes as a result of new studies from Timothy Padera, Rakesh K. Jain, and colleagues (Harvard Medical School, Boston, MA), who report that metastasis occurs through lymphatic vessels in the tumor margin and nearby normal tissue.

Cancer cells can metastasize by escaping a tumor through either blood or lymphatic vessels. Jain’s group questioned whether cancerous cells escape through lymphatic vessels within the tumor itself. In various transplanted and spontaneous tumors in mice, and in human tumors, markers of lymphatic vessels were found within the tumors, but functional analysis revealed that these vessels were not draining fluid.

Jain suggests that lymphatic vessels in tumors may collapse from the high pressure exerted by the rapidly proliferating cancer cells. The findings also act as a warning against the use of markers alone to identify functioning lymphatic vessels in tumors. “A combination of markers as well as functional studies will be necessary,” Jain says.

Although lymphatic vessels in the tumor were not functional, those in the margin of the mouse tumors were either normal in appearance or, in tumors overexpressing VEGF-C, larger in diameter than normal vessels. With increased size came



Lymphatic vessels (brown) in tumors are not functional.

increased lymphatic metastasis, suggesting that marginal lymphatics are sufficient for metastasis. “The larger size of these vessels increases the opportunity for cancer cells to escape,” says Jain. Thus, radiation therapy should be directed at tumor margins in addition to their centers, and VEGF-C may provide a good target for drug treatments. ■

Reference: Padera, T., et al. 2002. *Science*. 10.1126/science107.1420.

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