JOE BELCOVSON

Clodagh O'Shea and her team use a variety of techniques to investigate viral oncoproteins.

A research group in Italy is also heading in that direction. Physicist Alberto Diaspro, director of the nanophysics department at the Italian Institute of Technology in Genoa, is correlating AFM with super-resolution stimulated emission depletion (STED) microscopy to develop tools for topographical imaging, nanomechanical imaging, and measurements of cell stiffness[3]. In STED, researchers intentionally deactivate some fluorophores in part of the sample, which enhances the resolution in that area. Diaspro is optimistic that combining correlative-microscopy techniques with STED's nanoscale resolution will reveal valuable information about cellular nanostructures. The rapid image acquisition possible with STED also holds promise for measuring fast events, such as intracellular vesicle dynamics during secretion or neurotransmitter release, or cytoskeletal remodelling during cell motility.

FLUORESCENT BRIDGES

Researchers have long searched for a molecule that can bridge fluorescence and electron microscopy — a fluorescent molecule that also stains in EM images, for example. The ideal tool would be a genetically encodable molecular tag, small enough to penetrate fixed tissues better than awkward gold-labelled antibodies, but fluorescent and capable of delivering good contrast in EM. Two groups at the National Center for Microscopy and Imaging Research at the University of California, San Diego, are investigating a variety of molecules for use in more than one type of microscopy. Mark Ellisman, the centre's director, and biochemist Roger Tsien, who shared the 2008 Nobel Prize in Chemistry for developing the widely used green fluorescent protein (GFP) tag, often collaborate in what Ellisman calls "molecular painting" in the search for a reliable, genetically encodable tag to label proteins in EM. "We were looking for the GFP of electron microscopy," says Ellisman.

Last year, researchers from both groups revealed a molecular tool they had engineered called mini singlet oxygen generator (miniSOG), which is derived from a plant photoreceptor[4]. Singlet oxygen generator molecules are easy to see because they fluoresce in light microscopy and can be stained by diaminobenzidine for EM[5]. The group expressed proteins labelled with miniSOG to demonstrate its utility as a genetically encoded tag for protein targets in EM. "MiniSOG is an extremely good singlet oxygen generator, so we believe this one will get to single-molecule sensitivity," says Ellisman. "We're working on that now."

Meanwhile, miniSOG is already proving useful. Using a combination of microscopy techniques, molecular biologist Clodagh O'Shea and her colleagues at the Salk Institute for Biological Studies in La Jolla, California, are studying the puzzling observation that small viral oncoproteins seem to hijack cellular machinery to stimulate both viral and pathological cellular replication. According to O'Shea, her group wondered: "how do small viral oncoproteins win?" The group imaged infected cells by using miniSOG to label the adenovirus oncoprotein E4-ORF3 (ref. 6). Serial block-face scanning EM gave them reconstructed, three-dimensional views of infected cells. "The scanning electron microscope slices an infected cell from top to bottom in tiny 60-nm blocks," says O'Shea. The group also used electron tomography to make hundreds of 0.5-nm-thick computational slices through cells, and used specialized software to recreate cells from the computed slices. "Three-dimensional reconstructions show that E4-ORF3 assembles into a remarkable network of cables that weaves through the nucleus," she says. The images showed that the weave physically separates viral DNA replication domains from cellular nucleoli. In other words, she says, the viral oncoprotein self-assembles into a trap for tumour suppressors.

"We have been looking for the green fluorescent protein of electron microscopy."

A cousin of miniSOG is also on the horizon. Ellisman and former postdoc Alice Ting, now at the Massachusetts Institute of Technology in Cambridge, collaborated to create an enzyme called APEX that has enhanced singlet oxygen generator activity[7]. Ellisman stresses the importance of genetically encoded tags for miniSOG and APEX. "Both are molecules we can introduce genetically, and both will result in contrast in EM," he says.

Researchers continue to push the limits of correlative microscopy. "We are currently developing a methodology that combines CLIM with super-high-resolution fluorescence microscopy," says Stevens. "If successful, it will offer a new way of studying molecular interactions." Meanwhile, Ellisman and his colleagues are working on expressing two genetically encoded miniSOGs that have different colours. "This is what we call multicolour EM," he says. "The idea is that you would be able to do your dynamic light microscopy, then correlate a high-resolution subvolume down to the molecular scale. It's a hard project, but we know we'll succeed."

Bridging light and electron microscopy with genetically encodable fluorescent tags may one day be as routine as labelling with GFP is today. High-throughput methods applied to tissue sections may make super-resolution microscopy faster and easier. When that happens, two — or perhaps more — microscopes will definitely be better than one. ■

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CORRECTION

The Technology Feature 'Reading the second genomic code' (*Nature* **491**, 143–147; 2012) wrongly stated that Constellation and Genentech are collaborating to develop inhibitors of BET proteins and EZH2 chromatin-writers. Although they are working together on inhibitor development, the targets are not those mentioned.