SENSORS AND PROBES

A genetically encoded probe for EM

Electron microscopy welcomes a versatile genetically encoded protein tag.

Fluorescence microscopy experienced a 'green' revolution when GFP sent a surge of excitement for live imaging through the life sciences. Meanwhile, electron microscopy (EM) has been waiting patiently for the right probe to come along. EM offers brilliant high-resolution images of ultrastructure, but labeling molecules with specific antibodies is finicky business: the same permeabilization that grants access to targets also degrades fine structures in the cell.

Researchers led by Alice Ting at the Massachusetts Institute of Technology are pinning their hopes on a new generation of genetically encoded probes that avoid this trade-off. Their approach was to ask how the classic label horseradish peroxidase, which fails in highly reducing and calcium-poor environments such as the cytosol, could be made to work anywhere in the cell. "Initial experiments convinced us that this engineering would be extremely difficult, so we instead searched for heme peroxidases that are naturally active in the cytosol," says lead author Jeffrey Martell.

Horseradish peroxidase uses peroxide to convert 3,3'-diaminobenzidine into a product that can provide contrast for EM. "With guidance from Tom Poulos, one of our coauthors and a leading expert on heme peroxidases, we decided to test ascorbate peroxidase," says Martell. The peroxidase, abbreviated APX, hails from the pea plant. At first glance, it would seem to make a lousy probe: it exists as a dimer, making it more likely to perturb protein function in the cell, and its native substrate, ascorbate, is not very similar to diaminobenzidine.

The team compensated with several engineering improvements. To make an active monomeric form, they introduced mutations along the dimer interface with the help of the known crystal structure, prior mutagenesis results and sequence alignment to monomeric APX from corn. To compensate for weak heme binding, they modified the active site to resemble that from horseradish peroxidase, yielding faster kinetics and improved activity.

The final product is a tough little tag (about the size of GFP) dubbed APEX, which resists

STEM CELLS

HONING IN ON THE NICHE

Cultures of mouse spermatogonial stem cells on primary testis feeder cells model the *in vivo* niche.

A cell's surroundings can be critical in determining its fate. Adult stem cells, for example, which populate some mammalian tissues, are influenced by their microenvironment to either stay quiescent, divide or differentiate toward a particular lineage. Despite the interest and possible therapeutic potential of these cells, knowledge about their *in vivo* niche remains limited. One way to overcome this is to model the niche in the dish—as recent work has shown for spermatogonial stem cells (SSCs).

SSCs are responsible for producing spermatozoids and reside in the seminiferous tubules of the testis, where they are thought to interact with the basement membrane and with somatic cells known as Sertoli cells. Detailed study of SSCs and their niche has proven hard, partly because of their rarity and the difficulty in isolating them.

For years, SSCs have been identified retroactively, on the basis of their ability to re-initiate and maintain sperm production when transplanted into an infertile mouse host. But Mito Kanatsu-Shinohara and her colleagues, of Kyoto University in Japan, wanted to study the relationship between SSCs and their niche in greater detail, so they worked to develop a culture method in the likes of such an environment.

Kanatsu-Shinohara and her collaborators were inspired by the bone marrow—the best-studied stem cell niche in mammals. In this system, partial *in vitro* reconstruction of the *in vivo* niche is possible by culturing hematopoietic stem cells (HSCs) together with a stromal cell layer. HSCs form colonies of characteristic cobblestone morphology in which the cells maintain a steady state of self-renewal and differentiation resembling the *in vivo* situation. The number of cobblestone colonies also serves as a good estimate of the number of HSCs or progenitors that exist *in vivo*.

To mimic the basement membrane and cellular composition of the *in vivo* SSC niche, the researchers first cultured somatic cells from testis of infertile mice on laminin. They

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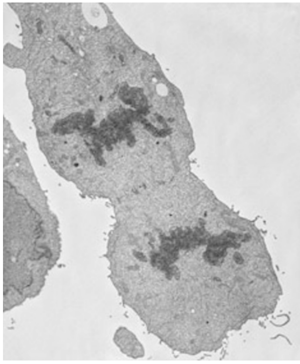

APEX probe fused to histone protein reveals fine chromosomal details in dividing cells. Image courtesy of T. Deerinck and J. Martell.

harsh fixation conditions and works in diverse compartments of the cell. Among other examples, APEX-tagged histones and intermediate filaments highlighted chromatin and cytoskeletal features at high resolution, and functional tagged versions of a mitochondrial ion transporter confirmed that it faces the interior of the organelle from its perch in the inner membrane.

APEX joins ranks with the genetically encoded reporter miniSOG, which converts diaminobenzidine for EM contrast by generating singlet oxygen in the presence of light. Although miniSOG is smaller than APEX (12 compared with 28 kilodaltons), the need for light limits how deeply thick tissue sections can be imaged. APEX has yet to be tested on tissue, but diaminobenzidine and peroxide diffusion are expected to allow deep imaging.

Both reporters can also be used for correlative light and electron microscopy. Fusing a fluorescent protein to the EM reporter (or using the mild inherent fluorescence of miniSOG) allows fluorescent features to be correlated and labeled sites to be located quickly by light microscopy before high-resolution electron scanning. The tools also carry the caveats of any genetically encoded reporter—they may alter the function of the protein that they report on—but unlike the partially genetically encoded ReAsH system, they do not suffer from nonspecific labeling.

The green-pea revolution may have just begun for EM microscopy. "We hope to develop improved versions of APEX with improved stability, heme binding, and reactivity toward diaminobenzidine," says Martell.

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RESEARCH PAPERS

Martell, J.D. *et al.* Engineered ascorbate peroxidase as a genetically encoded reporter for electron microscopy. *Nat. Biotechnol.* **30**, 1143–1148 (2012).

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then used these cultures as feeder layers onto which they added cells isolated from normal mouse testis enriched for SSCs. Two types of colonies arose from these cultures: one type seemed to gather in clumps; the other looked strikingly similar to cobblestone colonies in bone marrow cultures.

Kanatsu-Shinohara was excited when she saw cobblestone clusters in the SSC cultures—indeed, she knew these characteristic assemblies well from her years as a graduate student studying embryonic development of HSCs.

The group demonstrated that the number of cobblestone clusters served as a good estimate of SSC activity by doing parallel transplantation experiments. They then used this *in vitro* model to identify molecular players involved in stem cell homing and found two chemokines, GDNF and CXCL12, that played a role in this process—a finding that they also confirmed *in vivo*.

Though it is a big step forward, this culture setup can still be improved, explains Kanatsu-Shinohara. Currently, only primary testis cell feeders (containing a mixture of different testis somatic cells, including Sertoli cells) give rise to these cobblestone clusters. But the group wants to develop a Sertoli cell line that could be used instead, thereby making the entire process less labor intensive and more reproducible. They are also trying to identify additional surface markers expressed by SSCs that could be used to improve the isolation of these cells. Also on their 'to-do' list is the addition of a third dimension to the culture setup to better mimic the *in vivo* situation.

Ultimately, the team is interested in using SSCs as targets for germline modification of animals. A human-based version of this culture could also be useful for infertility treatments or for establishing disease models. Looking at how useful the cobblestone assay has been for understanding the bone marrow stem cell niche, one can't help but be excited for the bright prospects awaiting the spermatogonial field.

Erika Pastrana

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Kanatsu-Shinohara, M. *et al*. Reconstitution of mouse spermatogonial stem cell niches in culture. *Cell Stem Cell* **11**, 567–578 (2012).