performed saturation mutagenesis of the cysteine and fused the mutant LOV2 proteins to a singlet oxygen–sensitive infrared fluorescent protein. Finding LOV2 mutants that produced singlet oxygen was as simple as picking bacterial colonies that displayed the greatest decrease in infrared fluorescence after blue-light illumination.

Success came in the form of two colonies with a 70% decrease in infrared fluorescence. Subsequent improvement of the endogenous green fluorescence of the mutated LOV2 protein resulted in a mini singlet-oxygen generator (miniSOG) protein that produced singlet oxygen with more than ten times the efficiency of ReAsH (>100 times better than GFP chromophore) while still producing substantial fluorescence.

Shu *et al.* fused miniSOG to a variety of intracellular proteins expressed in mammalian cell culture, worms and mouse brain. They observed no perturbation of protein localization and normal fluorescence in all cell compartments, suggesting that flavin was never limiting and the fluorescence was not quenched during cell fixation. As expected, miniSOG was very efficient at photoconverting diaminobenzidine for electron microscopy labeling. In fact, it worked almost too well. Shu says, "Because the singlet oxygen generation quantum yield was so high, we had to limit the exposure time to make sure it didn't produce too many [diaminobenzidine] polymers and degrade resolution." Because miniSOG out-competes endogenous peroxidase for diaminobenzidine polymerization, background is very low.

Shu is optimistic the technology can be improved using fixation that avoids dehydration at room temperature. Through collaborations with colleagues he is also actively extending the use of miniSOG to other applications that directly use generated singlet oxygen.

Daniel Evanko

RESEARCH PAPERS

Shu, X. *et al.* A genetically encoded tag for correlated light and electron microscopy of intact cells, tissues, and organisms. *PLoS Biol.* **9**, e1001041 (2011).

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number of pseudogenes are actually translated into proteins. "We have always seen pseudogenes expressed," Harrow says, "but we have never seen translated ones." Of the ~10,000 pseudogenes present in the mouse genome, they saw evidence of translation for 19 of them. Validation of these expressed pseudogenes is ongoing, but Harrow sees it as reassuring that they only found a few, which indicates that the results are specific.

Of course, finding evidence of pseudogene translation does not say anything about the function of the protein; by common definition a pseudogene is a nonfunctional defective DNA segment that only resembles a gene. Choudhary plans to delve into analyzing the functional role of these newly discovered 'pseudoproteins' by looking at their localization and identifying their binding partners.

Having established the reliability of the pipeline, Hubbard plans to expand it to the human genome. "What we focused on up until now [in genome analysis] was entirely based on annotation by transcription," he says; "it is worthwhile trying to integrate the proteins."

Because some pseudogenes may not be 'pseudo' after all, it is possible that the same holds true for noncoding RNAs.

Nicole Rusk

RESEARCH PAPERS

Brosch, M. *et al.* Shotgun proteomics aids discovery of novel protein-coding genes, alternative splicing and 'resurrected' pseudogenes in the mouse genome. *Genome Res.* **5**, 756–767 (2011).

NEWS IN BRIEF

GENOMICS

Genome-wide map of the sixth base

Although 5-hyrdoxymethyl-cytosine (5hmC) is thought to be important for genome function in certain cells, a lack of tools has made profiling difficult. Xu *et al.* used a 5hmC-specific antibody for methylated DNA immunoprecipitation (MeDIP) and found distinct genome-wide 5hmC patterns in the mouse. The researchers saw 5hmC enrichment in gene bodies rather than at promoters and in intergenic regions. They also uncovered a role for the ten-eleven translocation (Tet) family of 5mC hydroxylases.

Xu, Y. *et al. Mol. Cell.* advance online publication (20 April 2011).

STEM CELLS

An eye in the Petri dish

Eiraku *et al.* report the formation of a primitive eye structure starting from a three-dimensional culture of mouse embryonic stem cells. The group grew embryonic stem cell aggregates on Matrigel and added factors that promote their differentiation toward retinal lineages. Under these conditions the cellular aggregates spontaneously formed hemispheral epithelial vesicles and progressively formed a structure reminiscent of the embryonic optic cup seen *in vivo*.

Eiraku, M. *et al. Nature* **472**, 51–56 (2011).

MOLECULAR ENGINEERING

Evolving RNA polymerase ribozymes

Wochner *et al.* describe the evolution of an RNA polymerase ribozyme capable of synthesizing up to 95-nucleotide RNA strands, much longer than the capabilities of natural ribozymes. They used water-in-oil emulsion technology with microbead display, linking ribozyme genes to multiple copies of the corresponding ribozyme; this allowed them to select ribozymes based on primer-extension capability.

Wochner, A. *et al. Science* **332**, 209–212 (2011).

IMAGING

High-throughput subcellular imaging of worms

High-resolution *in vivo* time-lapse assays in whole *Caenorhabditis elegans* worms require immobilizing the worms before imaging. Rohde *et al.* developed a system that allows doing this in a high-throughput manner using multiwell plates. They built an array of individually addressable cooling elements that fit into multiwell plates, and sequentially cooled down and imaged each worm using inverted epi-fluorescence microscopy. They used this technology to perform laser microsurgery of single neurons *in vivo*.

Rohde, C.B. *et al. Nat. Commun.* **2**, 271 (2011).

MOLECULAR BIOLOGY

A cellular system for epitope-tagged PrP

Goold *et al.* introduce an epitope tag into the cellular prion protein, PrP^{C}, expressed in a neuroblastoma cell line in which endogenous PrP has been knocked down by RNA interference; the tagged PrP^{C} supports prion replication and allows for the generation of infectious misfolded PrP^{Sc}. This approach allowed Goold *et al.* to distinguish newly formed PrP^{Sc} from inoculum-derived PrP^{Sc}; they observed very rapid infection of cells and found that prion conversion occurs primarily at the plasma membrane.

Goold, R. *et al. Nat. Commun.* **2**, 281 (2011).