chromosome 7. In this first trial only 0.5% survived. The difference in these two results showed that although removing the imprints on chromosome 7 alone is not sufficient for normal development, removal of the paternally imprinted regions on chromosomes 7 and 12 seems to be all that is required.

This interpretation was underscored by several tests ascertaining that the bi-maternal mice were normal: they performed similarly to wild-type mice in exercise and learning tests. The only difference Kono and his colleagues saw was lower body weight after birth and a smaller litter size.

The obvious question from this success is, 'What about bi-paternal mice?' Could the same strategy be used to investigate the imprinted regions in the maternal genome?

Kono's short answer is no, for two reasons. To date 13 regions are known to be maternally imprinted, and it would not be possible to delete so many genes in the paternal genome and retain a viable cell. In addition, unlike for maternal imprinting, which happens as the oocytes mature and enter meiosis, paternal imprinting is imposed on the fetal spermatogonia even before birth, long before the germ cells enter meiosis, and it would therefore not be possible to retrieve an imprint-free paternal genome.

Nevertheless, Kono is intrigued with the question of identifying the maternally imprinted regions essential for development. How this can be done will be the subject of future work—stay tuned.

Nicole Rusk

RESEARCH PAPERS

Kawahara, M. *et al.* High frequency generation of viable mice from engineered bi-maternal embryos. *Nat. Biotechnol.*, **25**, 1045–1050 (2007).

function or be toxic to cells, so the key to applying this technique is to find a toxicity threshold for each experiment. "There might be functions that these probes affect," cautions Discher, "but in the short term, the labeling of structural proteins is not devastating to the cell."

Used with caution, cysteines can also be engineered into proteins to query structural perturbations *in vivo* using this method. "You want to hide the cysteine in the quaternary structure or in a fold, so that it can become exposed," says Discher. "And that is the real challenge, to do that without perturbing function, but to do that so that hidden cysteines are exposed upon perturbation."

Recent advances in mass spectrometry made this analysis possible, and future improvements will allow more sensitive detection of probes. Discher points out that this study could not have been done just several years ago, and presently available technologies can detect the cysteine modification on thousands of peptides, especially from large structural proteins.

"The fluorescence gives you instant gratification," he points out, adding that now researchers "can bring it to the latest modern levels with mass spec."

Irene Kaganman

RESEARCH PAPERS

Johnson, C.P. *et al.* Forced unfolding of proteins within cells. *Science* **317**, 663–666 (2007).

NEWS IN BRIEF

MICROSCOPY

Super-resolution imaging goes multicolor

Recently several different techniques for super-resolution imaging of fluorescently tagged biological molecules have been described. Until now, only up to two colors could be imaged at one time. By finding new pairs of photoswitchable dyes for use with the previously described single-molecule photoswitching method, stochastic optical reconstruction microscopy (STORM), Bates *et al.* now demonstrate multicolor super-resolution imaging with three colors.

Bates, M. *et al. Science*, published online 16 August 2007.

GENOMICS

Saturating the mouse genome

Multi-lab efforts are underway to create a knockout mouse for each of its ~25,000 genes. Gragerov *et al.* enrich the available toolbox with a retroviral insertion strategy that leads to saturating random mutagenesis in 90% of the genome. With a targeted selection for the mutated embyonic stem cells, they can efficiently and rapidly identify knockout clones for almost any gene of interest.

Gragerov, A. *et al. Proc. Nat. Acad. Sci. USA* **104**, 14406–14411 (2007).

PROTEOMICS

Assessing ETD

Electron transfer dissociation (ETD) is a relatively new method for fragmenting peptides and proteins within a mass spectrometer to obtain more information about their molecular structures. With a systematic large-scale analysis, Good *et al.* demonstrate that ETD is highly complementary to the more traditional fragmentation method known as collision-activated dissociation (CAD), by showing that there was relatively little overlap in peptides identified by each of the methods.

Good, D.M. *et al. Mol. Cell. Proteomics*, published online 1 August 2007.

PROTEIN BIOCHEMISTRY

Genetic selection with tunable stringency

Selection schemes are often based on complementation of a metabolic defect in a model microorganism. Kleeb *et al.* now design flexible stringency selection by adopting strategies similar to those that control metabolic flux *in vivo*. By providing variable amounts of an enzyme that shunts a given metabolite down an alternative pathway in shikimate biosynthesis, they were able to select for bacterial dehydratases with activities over a 50,000-fold range.

Kleeb, A.C. *et al. Proc. Natl. Acad. Sci. USA* **104**, 13907–13912 (2007).

IMAGING AND VISUALIZATION

A molecular thermometer based on EGFP

Fluorescent proteins such as enhanced green fluorescent protein (EGFP) fluctuate between fluorescent and non-fluorescent states, a process known as blinking. Wong *et al.* observed that fluorescence blinking is strongly correlated to temperature and, as such, demonstrate that EGFP can be used as a molecular thermometer.

Wong, F.H.C. *et al. J. Am. Chem. Soc.* **129**, 10302–10303 (2007).