GENE REGULATION

A mouse with two mothers

By engineering bi-maternal mice from a wild-type and a mutant oocyte, researchers identify the contributions of paternal imprinting to normal development.

For mammals, female self-sufficiency is not an option when it comes to reproduction. Parthenogenesis, asexual reproduction, is precluded by the need for two separate genomes with individual imprinting, that is, the specific inactivation of genomic regions by epigenetic modifications such as histone or DNA methylation. Tomohiro Kono and colleagues from Tokyo University have had a long-standing interest in deciphering the role of imprinting in the developing embryo.

To elucidate the functional differences between the sperm and oocyte genome, they developed a technique that would spur the fantasy of every science fiction writer: they created a mouse from two females.

They reasoned that by taking an oocyte and eliminating the loci that are known to be inactivated in a haploid paternal genome they would create a maternal genome that imitates paternal features.

Figure 1 | Bi-maternal mice develop normally.

The first step was to create transgenic mice with deletions in these paternally imprinted regions. For this task they went back to earlier work describing genomic regions that are imprinted during spermatogenesis, which are essentially found on three chromosomes. They engineered mice with knockout mutations in two of these differentially methylated regions — one on chromosome 7, encompassing insulin-like growth factor 2 gene and *H19*, a gene for noncoding RNA, and the other on chromosome 21, covering an entire cluster of genes, important fetal growth factors among them. The third imprinted region on chromosome 9 they left intact as its gene is not involved in fetal development.

After two rounds of breeding they obtained females that were homozygous for the knockout on chromosome 7 and heterozygous for the deletion on chromosome 12 (mice homozygous for both loci were not viable), and collected the oocytes from the newborns. These oocytes were essentially imprint-free, but not able to grow. To mature them and induce meiosis, Kono's team had to transfer them into an enucleated oocyte from a wild-type mouse. With the haploid genome of the mutant oocyte in hand, they were finally ready to combine it with a haploid genome from normally growing oocyte and thus reconstruct a bi-maternal embryo.

They observed that almost 40% of their bi-maternal mice survived to adulthood, a rate similar to that seen with *in vitro* fertilized embryos (**Fig. 1**). This was a dramatic increase in efficiency compared to their earlier attempt in which they tried to create a bi-maternal embryo from a wild-type and a mutant oocyte that was only missing the imprinted region on

IMAGING AND VISUALIZATION

HIDE AND SEEK WITH CYSTEINES

To identify proteins that undergo structural changes in perturbed cells, researchers examined *in vivo* fluorophore labeling of normally shielded cysteines by fluorescence microscopy and mass spectrometry.

Many cellular activities are mediated by conformational changes in proteins or involve rearrangement of protein assemblies. These motions are now commonly investigated *in vitro* as well as at the single-molecule level. But Dennis Discher and his colleagues at the University of Pennsylvania wanted to develop an in-cell method to study these motions.

They were interested in studying molecular responses in cells under stress and initally developed their labeling technique in human red blood cells. The premise was to label cysteines with thiol-reactive probes in both stressed and unstressed cells. Then, differential labeling of proteins would indicate that under stress, previously buried cysteine residues became exposed and thus accessible to the fluorescent probe.

To implement this, the researchers reversibly lysed the red blood cells, loaded them with a thiol-reactive fluorophore, resealed them and then sheared a fraction of the cells using a fluid shearing device. After incubation to allow labeling, they lysed the cells again and quenched the unreacted label.

Immediately they could analyze these reactions by SDS-PAGE and also image the cells to see what parts of cells were differentially labeled. Having thus gotten an idea of what to expect, they then excised the bands of interest and subjected them to quantitative mass spectrometry analysis. "So you can run gels, and do a first analysis at the bench without sending every sample to a mass spec," summarizes Discher. "Mass spectrometry then positively identifies what proteins are labeled, and which cysteines are hit and by how much."

In the case of red blood cell shear stress, Discher's team found that the structural spectrin proteins were differentially labeled. This provided confirmation that the membrane cytoskeleton is important for this cell type's deformability under the stresses of blood flow. More specifically, they identified the cysteines that were labeled in the sheared cells, pinpointing which domains in spectrin unfold under stress. They also applied this method to study a more complicated system, the mesenchymal stem cell. Analysis of tensed and drug-relaxed cells provided a proteomic short-list of prominent structural proteins and also revealed differential labeling of several cytoskeletal proteins such as nonmuscle myosin and vimentin.

As with any label, the fluorescent probe can alter protein

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).