STEM CELLS

Many routes to pluripotency

Researchers observe that cells of the post-implantation mouse epiblast can revert to an embryonic stem cell–like state without the addition of exogenous genes.

Cellular reprogramming, these days, is all the rage. And although a lot of recent attention has been on the reprogramming of somatic cells to the pluripotent state—to yield induced pluripotent stem cells (iPSCs)—other types of reprogramming can be achieved in the culture dish as well.

The epiblast in the post-implantation mouse embryo develops from the inner cell mass and forms the tissues of the embryo proper. It was shown some years ago that it is possible to derive Epi–stem cells (Epi-SCs) from this structure. Epi-SCs are pluripotent *in vitro*, but they do not contribute to mouse chimaeras and their epigenetic and gene expression patterns are different from those of mouse embryonic stem cells (mESCs).

Azim Surani and colleagues, at the University of Cambridge, now show that cells of the post-implantation mouse epiblast can revert to a mESC-like state without the addition of exogenous genes. They came to this observation from studies of primordial germ cells (PGCs), which are derived from the epiblast by yet another reprogramming event during development. "We know that epigenetic reprogramming of the epiblast happens *in vivo* in the context of germ cells," explains Surani, "so we thought it might be able to go back to a more embryonic stem cell–like state as well."

To do this, the researchers dissected the epiblast of postimplantation mouse embryos (E5.5–E7.5) away from the region that contains PGCs and PGC precursors and, critically, dissociated it with trypsin to a single-cell suspension. They used embryos that carry a fluorescent reporter expressed in the inner cell mass, embryonic stem cells and PGCs, but not in the epiblast or in Epi-SCs. Upon culture of these dissociated cells under conditions used for mESC derivation, they observed green cells after a few weeks; these could be passaged to attain reprogrammed embryonic stem cell–like cells or rESCs. Furthermore, the researchers used this approach to obtain rESCs from established Epi-SC lines as well, though in this case single-cell dissociation was no longer critical.

The rESCs have patterns of gene expression and of DNA methylation closer to those of mESCs than of Epi-SCs or epiblasts, show reactivation of the inactive X chromosome and, crucially, contribute to mouse chimaeras, including the germline. The researchers also showed that derivation of these cells requires STAT-3 signaling. "I think what this tells us is that there are two ways to reprogram the epiblast: by LIF-STAT3 signaling or by an alternative mechanism through specification into the PGC," says Surani.

Like the Epi-iPSCs derived earlier this year which in contrast required the expression of exogenous genes, rESC derivation could prove a good model system for studying certain aspects of somatic cell reprogramming. Some of the epigenetic changes that take place, such as reactivation of the inactive X chromosome, are similar to those encountered in making iPSCs, so that rESC derivation

MICROARRAYS

LOOKING FOR A REACTION

New chemical microarrays capture a comprehensive snapshot of the various enzymatic activities contained within a biological sample.

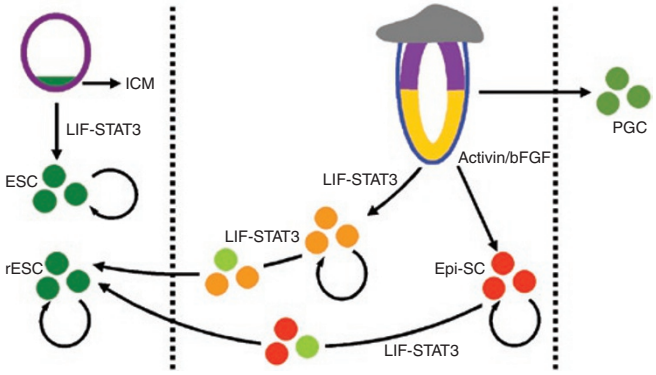
Even as next-generation sequencing platforms have delivered rapid and accurate genomic sequencing to scientists, the process of annotation and functional characterization of genes has lagged behind, and the gap continues to widen.

"Every single-cell or environmental genomic project has added a huge number of putative enzymes, the functions of which are often unknown and at best deduced from sequence comparison," says Manuel Ferrer of the Institute for Catalysis in Madrid. "For example, from the 27 million open reading frames found in the Global Ocean Sampling Project—coding 5.7 million nonredundant protein sequences—only a few dozen have been characterized."

In an effort to decouple the science of metabolomics—the full characterization of metabolic processes taking place in a biological specimen—from genomic analysis, Ferrer and colleagues, including Peter Golyshin of Bangor University in the UK, developed an assay based on enzyme-substrate interactions. They built what is essentially a metabolite microarray, in which a large collection of known metabolites and enzymatic substrates are tethered onto a slide with a specially designed linker and then exposed to lysate derived from cells of interest. Each target molecule incorporates a Cy3 dye label that is subject to intramolecular quenching; upon enzymatic binding and catalysis, the substrate is released, and the quenching is relieved. "The fluorescent signal obtained for each spot provides a quantitative measure of the enzyme activity present in [a cellular] lysate, and bioinformatic analysis of the array results provides a global overview of the metabolic network of the cell—the 'reactome'—at the moment of sampling," explains Ferrer. The substrate linkers are also designed to physically trap reactive enzymes so that array results of interest can be investigated further via mass spectrometric analysis of proteins captured by a metabolite or subset of metabolites.

A key advantage of this array approach is that, by focusing on biochemically relevant interactions, it becomes essentially species-independent. "The molecules that the array contains are universal for all forms of life; in fact, the metabolites collectively represent the central metabolic pathways of all cellular systems," says Ferrer. "For this reason, it may be useful for any kind of sample, ranging from single cells, to environmental samples, to tissues, blood samples and so on."

In an initial trial, the team characterized the 'reactome' of the bacterium *Pseudomonas putida* and observed a strong correlation between their enzyme-substrate profile and predictions based on this species' annotation in the Kyoto Encyclopedia of Genes



Schematic depicting pluripotent cell types that can be derived from the mouse embryo. ICM, inner cell mass; ESC, embryonic stem cell; rESC, reprogrammed ES cell-like cell; EpiSC, epi-stem cell; PGC, primordial germ cell. Image adapted from *Nature*.

could be useful to study the role of certain epigenetic mechanisms and other modifiers in the reprogramming process.

The work also has implications for studies of human embryonic stem cells (hESCs), which are thought to resemble mouse Epi-SCs. "There are a lot of people trying to revert hESCs to a more mESC-like state," Surani says. "Hopefully this might give some encouragement."

Natalie de Souza

RESEARCH PAPERS

Bao, S. *et al.* Epigenetic reversion of post-implantation epiblast to pluripotent embryonic stem cells. *Nature* **461**, 1292–1295 (2009).

and Genomes (KEGG)—the source for many of the substrates used on their array. However, they could also directly assign functions to dozens of hypothetical proteins whose existence had been predicted based solely on sequence data and to refine the functional annotations of many previously characterized enzymes.

Investigating the ecosystem in an environmental sample increases the metabolomic challenge by orders of magnitude. "One gram of soil can contain millions of reactions, enzymes and microbes," says Ferrer. However, because of its species-independence, the reactome chip proved useful for culling informative metagenomic data from diverse environments. For example, they found that a mineral-rich geothermal pool predominantly contained species with heightened enzymatic capacity for iron and sulfur oxidation whereas a sample of heavily polluted water from the Barents Sea was highly enriched for species with adaptations for efficient petroleum degradation and hydrocarbon use.

Other potential applications for this technology could include characterization of physiological changes resulting from cancer or infection, or analysis of metabolic alterations in transgenic plants, and Ferrer is keen to forge new collaborations. "We believe that the technology offers many possibilities for doing both basic and applied research," he says.

Michael Eisenstein

RESEARCH PAPERS

Beloqui, A. *et al.* Reactome array: forging a link between metabolome and genome. *Science* **326**, 252–257 (2009).

NEWS IN BRIEF

CELL BIOLOGY

3D cell culture on paper

Systems for three-dimensional (3D) cell culture exist, but controlling the distribution of oxygen and nutrients remains a challenge. Derda *et al.* now report a simple approach for constructing paper-supported 3D cell cultures. They added a hydrogel precursor solution containing suspended cells to a small piece of paper and gelled it in place. This allowed them to stack multiple layers of gelled paper to generate a 3D culture system. These stacks can also be readily destacked to examine the cells in the center of the culture.

Derda, R. *et al. Proc. Natl. Acad. Sci. USA* **106**, 18457–18462 (2009).

RNA INTERFERENCE

Predicting targets of microRNA

MicroRNAs regulate gene expression by binding to mRNAs and inhibiting translation. Algorithms for discovering microRNA-mRNA pairs have been developed but are prone to inaccuracies, so Ritchie *et al.* set out to develop an alternative approach for microRNA target discovery based on comparing the expression levels of microRNAs and mRNAs in mouse and in human, across multiple tissues. They used the conserved microRNA signatures to predict thousands of microRNA targets.

Ritchie, W. *et al. PLoS Comput. Biol.* **5**, e1000513 (2009).

GENOMICS

Human methylome at base pair resolution

After *Arabidopsis thaliana, Homo sapiens* is the second species to have its DNA methylome sequenced in its entirety. Lister *et al.* subjected bisulfite-converted genomic DNA to high-throughput sequencing and mapped all methylated cytosines in the genomes of human embryonic stem cells, where they found 25% of methylcytosine in non-(C+G) context, and in fetal lung fibroblasts, where non-(C+G) methylation had disappeared.

Lister, R. *et al. Nature* advance online publication (14 October 2009).

PROTEIN BIOCHEMISTRY

Site-specific histone acetylation

Modifications in the cores of histones have important roles in regulating the structure and function of chromatin. Neumann *et al.* describe an approach to recombinantly generate site-specifically acetylated histones using an evolved aminoacyl-tRNA synthetase and $tRNA_{CUA}$ pair that facilitates the incorporation of acetyl-lysine in response to an amber codon. This method allowed the researchers to investigate the mechanistic role of Lys56 acetylation, a highly conserved modification in histone H3, in detail.

Neumann, H. *et al. Mol. Cell* **36**, 153–163 (2009).

GENE REGULATION

Knocking down microRNA in chick embryos

There is great interest in studying the function of microRNAs in controlling gene expression during embryonic development. McGlinn *et al.* now describe an approach for knocking down microRNA function in developing chick embryos by using antagomiRs, engineered antisense oligonucleotides, to specifically target Hox genes. The researchers used this method to explore the function of miR-196 in regulating Hox genes central to axial skeletal patterning.

McGlinn, E. *et al. Proc. Natl. Acad. Sci. USA* **106**, 18610–18615 (2009).