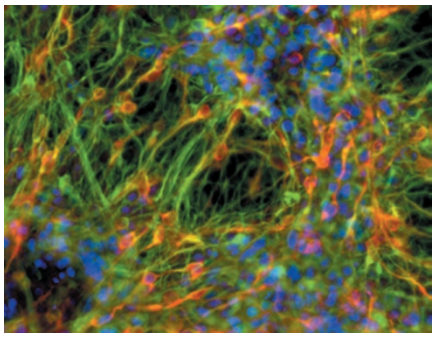
STEM CELLS

Predicting neurogenesis

Expression of a microRNA cluster predicts whether or not a particular human pluripotent stem cell line will differentiate well into neurons.

One of the difficulties that plague researchers working on human pluripotent stem cells is that cell lines appear to vary in their propensity to differentiate. Just because a differentiation method works well to make neurons from one cell line, for instance, does not mean that it will work well for another line. So what is one to do? Throw up one's hands and work on HeLa cells? Painstakingly optimize differentiation protocols for each cell line? A more elegant approach might be to predict which cell line is inclined to differentiate into the cell type of interest.

Lorenz Studer and his colleagues at the Sloan-Kettering Institute in New York have been considering this problem for a long time. Before human induced pluripotent cells (hiPSCs) were even reported, they were studying human embryonic stem cell (hESC) differentiation to dopaminergic neurons, and they finally became convinced that there are consistent differences between lines. It is worth noting that this in itself is not trivial. As Studer cautions, "a big problem of such studies is that any differences might just be a matter of conditions of the culture at that moment in time. Any differences need to be consistent over a range of passages, multiple replicates, subcloning of the cells and so on, before you can start to think that the differences are intrinsic to the cells."



Dopaminergic neuron precursors derived from human iPSCs. Image courtesy of L. Studer.

But having concluded that they were indeed seeing such differences in stem cell behavior, the researchers then wanted to know what lay beneath and whether they could identify markers predictive for neural differentiation. And then, when the derivation of hiPSCs was reported, the potential value of such predictors became even more obvious. "As the methods improve, it's not too difficult to make ten clones of iPSCs from fibroblasts, but then which of them are you going to take forward?" asks Studer. What is more, because one of the very interesting applications of hiPSCs is the study of disease phenotypes in cells derived from multiple people, it is critical that the experimental design allows one to examine cellular differences that are specific to the disease or to the genotype and not merely the consequence of intrinsic variability of the lines.

Studer and colleagues initially looked at four hESC lines, using microarrays to examine global gene expression and microRNA expression patterns, searching for clues. They noticed that the most neurogenic cell line preferentially expressed genes that are associated with so-called epiblast stem cells in the mouse. Conversely, the least neurogenic cell line strongly expressed the *miR-371* microRNA cluster, the ortholog of the mouse *miR-290* cluster, which is thought to mark classical embryonic stem cells in the mouse. This initial analysis therefore already gave the researchers a hint that underlying

STEM CELLS

SWIFT, FLEXIBLE KNOCKOUTS

Researchers produce a mouse embryonic stem cell library along with convenient vectors.

Scientists eager to understand the function of a gene often get stuck in drudgery: putting the desired alleles into mouse embryonic stem cells can easily take the better part of year. Now, scientists at the Wellcome Trust Sanger Institute have created a welcome resource (Skarnes *et al.*, 2011). In addition to making mouse embryonic stem cell lines for interrogating 9,000 genes, they designed vectors that can help researchers quickly put additional genes under experimental control. The work is part of an international effort to target each of the 20,000 or so mouse genes.

The vectors used in the project incorporate what is called a 'knockout-first' design. The engineered allele is initially inactive, but can be reactivated and inactivated again by a series of recombinases, allowing researchers to choose the developmental stage or the tissue in which to study the allele. "You make the derivative alleles you want by crossing the mice," explains William Skarnes, project leader for the high-throughput gene knockout program. For example, to inactivate gene function in hippocampal neurons, mice carrying the knockout-first allele would be bred with mice expressing the appropriate DNA recombinase in hippocampal neurons.

What is more, the alleles can also be converted to reporter genes, and vectors enable mix-and-match elements: researchers can easily replace the default *lacZ* reporter gene with any other reporter. A selection cassette can be swapped with others to permit multiple alleles to be targeted in the same cell. Barry Rosen, a coauthor, conceived the modular strategy, says Skarnes. Not only does it make the vectors flexible in the present, it also "future-proofs the resource," he says, allowing researchers to incorporate genetic tools that have yet to be invented.

To design the vectors, the researchers began with a computer program that scoured the mouse genome, figuring out where to place selection cassettes and other machinery as well as identifying critical exons whose removal would shift a gene's reading frame. This approach worked for about 60% of protein-coding genes but not for smaller genes containing only a single exon. However, says Skarnes, his team has since developed a new design that overcomes this limitation.

But designing vectors that work for thousands of genes is not the same as making thousands of vectors and putting them into thousands of embryonic stem cell cultures. For that, the researchers created an extremely efficient vector-assembly process

the variable differentiation behavior there may be some differences in the pluripotent state.

They went on to examine many more cell lines, including both hESC and hiPSC lines, and found, in a retrospective analysis but importantly also prospectively, that expression of the *miR-371* cluster is predictive of neural differentiation. In other words, the researchers could predict the neural differentiation behavior of cell lines, via two entirely different differentiation protocols, purely on the basis of expression of *miR-371*. Specifically, they saw that lines with low *miR-371* expression are more neurogenic. Expression of this marker could also predict which stem cell lines could give rise to dopaminergic neurons that stably engraft *in vivo*.

Although Studer and colleagues had clearly identified a practically useful marker, they were also interested in the underlying biology. "We didn't really know when we began," Studer says, "if we would find random markers that for whatever reason mark a certain behavior or if there would be some logic behind it that would tell us why the cells behave differently." As it turns out, understanding the role of the *miR-371* cluster in differential stem cell behavior is somewhat complicated. The marker distinguishes between cell lines with different requirements for bone morphogenic protein (BMP) signaling in neural induction, but the finer details of its role will require additional studies.

In the meantime however, it should prove practically useful to identify human pluripotent stem cell lines that enthusiastically make neurons, and suggests that similar markers may be identified that are predictive for differentiation to other cell types.

Natalie de Souza

RESEARCH PAPERS

Kim H. *et al.* miR-371-3 expression predicts neural differentiation propensity in human pluripotent stem cells. *Cell Stem Cell* **8**, 695–706 (2011).

so that multiple steps could be conducted in 96-well plates without the need to grow and select clones between steps. They subjected a bacterial artificial chromosome to three rounds of modification, purified the resulting plasmid and passed it through recombinase-expressing bacteria to generate an intermediate targeting vector. The final vector is assembled, conveniently, *in vitro*. Finally, they electroporate the vectors into a mouse embryonic stem cell line that contributes strongly to the germline and other tissues in chimeric mice (Pettitt *et al.*, 2009)

The technique should work for other mouse strains and even other species, says Skarnes. He and his colleagues are currently using this system to eliminate both copies of genes in embryonic stem cells to study gene function in a model cell.

But the real payoff will come not from engineering cells but from studying mice derived from them. That is why Skarnes is particularly excited that several international government bodies are supporting this task. "Once the cells are converted into mice," he says, "we, [scientists,] can start the real work, which is to understand gene function."

Monya Baker

RESEARCH PAPERS

Pettitt, S. J. *et al.* Agouti C57BL/6N embryonic stem cells for mouse genetic resources. *Nat. Methods* **6**, 493–495 (2009).

Skarnes, M.C. *et al.* A conditional knockout resource for the genome-wide study of mouse gene function. *Nature* **474**, 337–342 (2011).

NEWS IN BRIEF

MODEL ORGANISMS

Zinc-finger nucleases for gene correction *in vivo*

There a great deal of interest in the use of zinc-finger nucleases for tailored genome engineering, but they have not yet been used for genome modification *in vivo*. Li *et al.* now use zinc-finger nuclease–mediated targeting of a promoter-less DNA fragment to correct mutations in a mouse model of hemophilia B. They intraperitoneally injected a hepatotropic adeno-associated viral vector to deliver the nuclease and the therapeutic fragment, and observed sufficiently effective gene targeting to restore functional clotting in the mouse.

Li, H. *et al. Nature* advance online publication (26 June 2011).

MASS SPECTROMETRY

Introducing the Q Exactive

Michalski *et al.* introduce the Q Exactive, a benchtop mass spectrometer with many beneficial advantages for proteomics research. This instrument combines quadrupole and Orbitrap analyzers, allowing multiplexed operation for single-stage and tandem mass spectrometry. Compared to current top-of-the-line Orbitrap instruments, the Q Exactive also offers high mass spectrometric resolution, identifies more peptides in a single run and is faster and easier to use.

Michalski, A. *et al. Mol. Cell. Proteomics* advance online publication (3 June 2011).

STRUCTURAL BIOLOGY

Combined solution and solid-state NMR spectroscopy

Bertini *et al.* report a method for investigating the structure of large proteins by nuclear magnetic resonance (NMR) spectroscopy both in solution and in solid state without changing the sample tube. They first performed solution-state NMR measurements on the protein apoferritin. Then, by spinning the sample tube at ultracentrifugation speeds, the protein sedimented on the tube walls, allowing them to make solid-state measurements. The method is applicable to proteins larger than about 100 kilodaltons.

Bertini, I. *et al. Proc. Natl. Acad. Sci. USA* **108**, 10396–10399 (2011).

MOLECULAR ENGINEERING

A minimalist nuclear pore

Disordered Phe-Gly domains of nucleporins are thought to constitute the selectivity filter at the nuclear pore. Kowalczyk *et al.* report a biomimetic nuclear pore complex capable of selective protein transport. The minimalist structure consisted of a silicon-based nanopore coated with nucleoporin Phe-Gly domains. Stringency of selectivity depended both on nanopore diameter and the nucleoporin of choice, revealing intrinsic differences between nucleoporin function at the selectivity barrier.

Kowalczyk, S.W. *et al. Nat. Nanotechnol.* **6**, 433–438 (2011).

IMAGING

Fluorescent cell biolasers

Lasers emit light through optical amplification of input electromagnetic energy. This is achieved through stimulated photon emission by an appropriate "gain medium" inside a highly reflective optical resonator. By pumping single fluorescent cells with brief optical pulses in a mirrored biconcave microcavity, Gather and Yun could stimulate the emission of bright directional laser beams without affecting cell viability. The concept could enable new techniques for cellular and tissue imaging.

Gather, M.C. & Yun, S.H. *Nat. Photonics* **5**, 406–410 (2011).