GENETICS

A toolset for the proficient geneticist

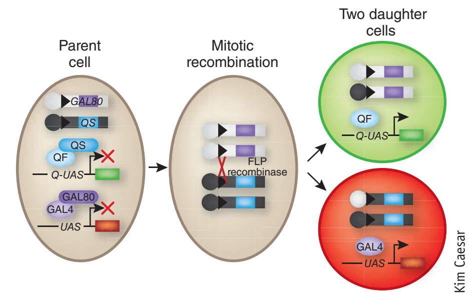
New strategies expand the genetic toolkit for transgene expression, lineage tracing and mosaic analysis of gene function in flies and mammalian cells.

Body tissues comprise many different cells that originate from specific precursors. Understanding this complex organization of cells requires methods to label small cellular populations from their birth and throughout differentiation as well as to precisely manipulate them. The most effective and versatile method among these is the use of engineered transgenes whose expression can be turned on or off in a controlled manner.

Binary expression systems are one of such strategies that allow fine control of transgene expression in model organisms. In *Drosophila melanogaster*, the archetypal binary expression system is GAL4-UAS, in which the yeast transcription factor gene *GAL4* is under the control of a promoter of interest. GAL4 in turn activates a second exogenously inserted transgene bearing an upstream activating sequence (UAS). This binary system can be additionally regulated by GAL80, a GAL4 repressor.

Liqun Luo's group at Stanford University was in search of additional repressible binary expression systems that could be used as alternatives or in combination with the GAL4 system. They found a good candidate in the bread mold *Neurospora crassa* and named it the Q system. The Q system consists of a transcriptional activator, QF, which binds to a specific sequence, *Q-UAS*, as well as its repressor, QS. In flies, QS activity can be blocked by quinic acid added to the fly's food, providing another level of control over transgene expression. Whereas other binary expression systems have been modified to achieve similar levels of control, "this one has all the bells and whistles built into it," explains Luo.

One common application of the GAL4 system is to create mosaic animals via mosaic analysis with a repressible cell marker (MARCM). In MARCM, cell division is essentially coupled with the loss of a



Scheme for coupled MARCM, one of the applications of the Q system. FLP recombinase–mediated mitotic recombination followed by specific chromosome segregation produces two distinct daughter cells lacking QS or GAL80 and thus capable of expressing different transgenes.

transcriptional repressor in one of the two daughter cells, thus allowing marker expression solely in this cell and all of its progeny. MARCM has been widely used for lineage analysis, neural circuit tracing and high-resolution mosaic analysis of gene function. Luo's group, the developers of MARCM,

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STEM CELLS

WHERE DO YOU COME FROM?

Live-cell time-lapse imaging of somatic cells undergoing reprogramming raises interesting questions about the mechanism of the process.

One of the problems that continues to both bedevil and tantalize those studying the reprogramming of somatic cells to induced pluripotency is the very low efficiency of the process. Now researchers at the Harvard Stem Cell Institute bring live-cell imaging to bear on the question of what happens during reprogramming.

The project arose from a collaboration. "I was working in a yeast lab doing live-cell imaging," explains Iftach Nachman, co–first author with Zachary Smith on the paper, "and I was talking with Alex [Meissner, senior author on the paper], about collaborating. When they started to describe the reprogramming system, that only 1% of the cells do this, we decided to follow this process with live-cell imaging to try and understand it."

The researchers used mouse fibroblasts from the so-called secondary reprogramming system, in which viruses carrying the reprogramming factors Oct4, Sox2, Nanog and c-Myc are stably integrated into the fibroblast genome and are expressed in response to doxycycline treatment (it is worth noting that reprogramming efficiencies in the secondary system are typically much higher than when the factors are newly delivered to somatic cells).

It was necessary to tag the cells for these experiments. To do this, Smith and colleagues introduced doxycycline-inducible fluorescent protein constructs into the cells and seeded the tagged cells into a background of untagged ones. This allowed them to follow specific lineages over the course of the reprogramming experiment. They turned on the reprogramming factors and the fluorescent protein with doxycycline, took images every 6–12 hours for two weeks, fixed the cells, stained them for markers of pluripotency (Nanog and E-cadherin) to identify the induced pluripotent stem cell (iPSC) colonies and then examined the time-lapse images to trace back to the cell from which each of the colonies arose.

They could trace many of the iPSC colonies back to a subset of rapidly dividing cells with a characteristic size and shape. Notably, they observed this accelerated proliferation as early as the first cell division and consistently saw it in all cells in the lineage. "If you follow a single yellow cell and the cells that come from it, all of them seem to do the same thing," Nachman says. In contrast to previous work suggesting that reprogramming is a stochastic process that will occur in all cells given sufficient time, this observation implicates an early decision in the ability to reprogram. The experiments cannot, however, distinguish between an early stochastic event versus the existence of a predetermined subset of cells that are in

now shows that Q system–based MARCM can be used with GAL4-MARCM to independently mark and manipulate two populations of cells and study their cell-cell interactions.

Using this 'coupled MARCM' approach, they traced lineages and studied cell division patterns of neuroblasts in the fly olfactory system and of cellular populations of the wing imaginal disc. They show that combining these two binary systems into logic gates creates a whole battery of new expression patterns that can help gain genetic access to specific cells.

Without a doubt, one of the major powers of the GAL4 system is its ability to manipulate many cell types through thousands of *GAL4* lines generated by the fly community over the years. Similarly, the broader community should now be encouraged to generate large numbers of *QF* lines with different expression patterns. A current limitation of the Q system is the inability to generate ubiquitously expressing *QF* transgenic flies, a hurdle that Luo hopes will soon be overcome.

Albeit with some differences, the Q system also works in mammalian cells. Future work is needed to make this tool conducive for mouse transgenesis, and it will be equally interesting to know if it can be applied to worms or zebrafish. Luo is optimistic that researchers will not be discouraged by the high number of transgenes required for some of the Q system applications—eight for coupled MARCM. "If it can do things uniquely, people will overcome their high energy barrier and delve into it," he adds with confidence.

Erika Pastrana

RESEARCH PAPERS

Potter, C.J. *et al.* The Q system: a repressible binary system for transgene expression, lineage tracing, and mosaic analysis. *Cell* **141**, 536–548 (2010).

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some way primed for reprogramming. What is more, any such early event in the induced cells is clearly not sufficient because continued expression of the reprogramming factors is required during this period.

The researchers also realized that they could not trace the origin of a subset of iPSC colonies. They saw these arise, in an as-yet-undefined manner, at 4–8 days after induction of the cells. As this is the time period when the initially formed colonies undergo compaction, it is possible that these 'satellites' arise from cells that detach from other colonies. This underscores the fact that changes in reprogramming efficiency, typically measured as the number of iPSC colonies per number of starting cells, could artifactually report other changes in the cultures, either in cellular properties or in the way that the cells are handled.

Sometimes you can learn a lot by just taking a look, but it is fair to say that imaging the reprogramming process has raised more questions than it has answered. They are fascinating questions, though, and will undoubtedly seed more detailed and targeted studies. Live imaging is catching on in many different domains of stem cell research and will surely continue to enable scientists to both ask and answer questions about these most dynamic and interesting of cells.

Natalie de Souza

RESEARCH PAPERS

Smith, Z.D. *et al.* Dynamic single-cell imaging of direct reprogramming reveals an early specifying event. *Nat. Biotechnol.* **5**, 521–526 (2010).

NEWS IN BRIEF

GENOMICS

The splicing code, decoded

For many years, researchers have attempted to define the combinatorial rules that control alternative splicing. Barash *et al.* now report a code, implemented in a web tool, that predicts tissue-specific alternative splicing with high accuracy. To construct the algorithm, they analyzed a large amount of data profiling alternatively spliced exons in diverse mouse tissues, known RNA binding sites and sequence motifs, exon-intron organization characteristics, evolutionary conservation and RNA fold structure.

Barash, Y. *et al. Nature* **465**, 53–59 (2010).

STEM CELLS

Making mechanosensitive sensory hair cells

Damage to mechanosensitive sensory hair cells found in the mammalian inner ear can result in permanent hearing loss and balance problems because these cells do not regenerate. Oshima *et al.* report a protocol to generate such cells, which could eventually be used in therapies. Starting with embryonic stem cells and induced pluripotent stem cells, they identified conditions for differentiation into hair cell–like cells that exhibited characteristic hair cell morphologies and were responsive to mechanical stimulations.

Oshima, K. *et al. Cell* **141**, 704–716 (2010).

NEUROSCIENCE

A bold application of optogenetics

Functional magnetic resonance imaging measures brain activity by detecting blood oxygenation level–dependent (BOLD) signals. For a long time, the link between BOLD signals and neural firing has been hard to demonstrate. Using optogenetic tools to excite specific sets of neurons, Lee *et al.* now show that neuronal activity elicits BOLD signals. The unification of these two powerful methods will open new possibilities for mapping large-scale neural circuits in the brains of live animals.

Lee, J.H. *et al. Nature* **465**, 788–792 (2010).

BIOSENSORS

Digital ELISA

The enzyme-linked immunosorbent assay, ELISA, is not sensitive enough to detect proteins found in very low concentrations in biological samples. Rissin *et al.* describe a 'digital ELISA', which can be used to detect proteins in serum at concentrations as low as 10^{-15} M. Using an on-bead ELISA format, they capture proteins of interest and distribute the beads into femtoliter-volume well arrays, where they image them by fluorescence microscopy. The percentage of fluorescent beads is proportional to the concentration of the protein in the sample.

Rissin, D.M. *et al. Nat. Biotechnol.* **28**, 595–599 (2010).

BIOPHYSICS

High-throughput single-molecule force spectroscopy

Typical single-molecule force spectroscopy procedures require substantial time and effort to acquire multiple measurements. Halvorsen and Wong describe a centrifuge force microscope in which the instrument components and sample—consisting of thousands of beads bound to a coverslip via a DNA tether and receptor-antigen pair—are on a rotating arm. Centrifugal force pulls the beads from the surface, and visualizing the rupture events yields thousands of parallel measurements of receptor unbinding.

Halvorsen, K. & Wong, W.P. *Biophys. J.* **98**, L53–L55 (2010).