TECHNOLOGY

Cre-ative mouse mosaics

Fly geneticists have been performing a nifty trick for some time now in which they generate patches of cells that are homozygous for a mutation or a marker gene in heterozygous flies. These so-called genetic mosaics — organisms of more than one genotype — have been instrumental in cell-lineage and cell-fate determination studies of fly development, and have been created by using the site-specific recombination system, Flp/*FRT*, to induce recombination during mitosis (see link to animation for more). This approach has also been tested in mice, without much success, but now Pentao Liu and colleagues report a Cre/*lox*P-based strategy that induces mitotic recombination in mouse embryonic stem (ES) cells at reasonable frequencies and in genomic regions that are less amenable to this type of event.

Liu *et al.* began by generating two recombination cassettes that each contain complementary, but non-functioning, halves of a human *HPRT* minigene that are flanked by *lox*P sites, which they targeted to various allelic chromosomal regions in an *Hprt*-deficient ES cell line. When Cre recombinase is expressed in doubly targeted cells, the 'floxed' cassettes on non-sister chromatids can recombine to create a functioning *HPRT* gene, allowing cells with recombinant chromatids to become HAT (hypoxanthine, aminopterin and thymidine) resistant (HAT^r). When the authors targeted these cassettes to a chromosome 7 locus (*D7Mit178*), they achieved tenfold higher recombination frequencies than those reported in previous studies. And to check that the recombinant chromatids segregate away from each other in mitosis — so-called X segregation, which produces homozygous mutant cells — the authors assayed the methylation status of a nearby imprinted gene, *Snrpn*, and found that all HAT^r cells were uniparental distal to the recombination event at *D7Mit178*.

But such recombination frequencies were not achieved in other genomic regions. When the cassettes were targeted to *Wnt3* and to *D11Mit71* on chromosome 11, recombination frequencies dropped considerably, but increased when *cre* was constitutively expressed for up to eight days. Liu *et al.* also increased recombination frequencies by replacing each single *loxP* site in the chromosome-7 recombination cassettes with three *lox* variants. Although this modification improved mitotic recombination frequencies, it also reduced the apparent incidence of X segregation, probably because the extra *lox* sites mediate a second exchange between sister chromatids.

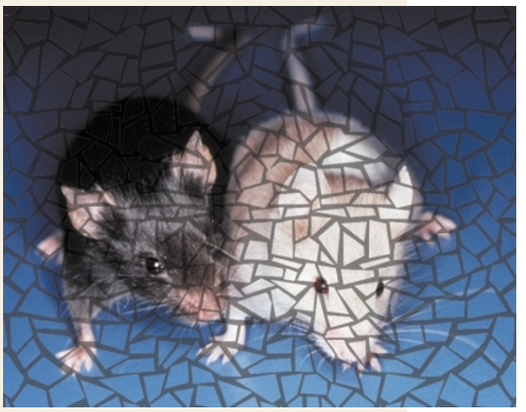

Photo by Karen Moore, courtesy of Nancy Jenkins.

Although this approach has yet to be tested *in vivo* — for example, by introducing recombined mutant clones into wild-type blastocysts or by generating them *in vivo* by the spatially and temporally controlled expression of *cre* — it will no doubt help mammalian geneticists to piece together new pictures of gene function.

Jane Alfred

References and links

ORIGINAL RESEARCH PAPER Liu, P. *et al.* Efficient Cre–*lox*P-induced mitotic recombination in mouse embryonic stem cells. *Nature Genet.* **30**, 66–72 (2002)

FURTHER READING Adams, D. J. & Bradley, A. Induced mitotic recombination: a switch in time. *Nature Genet.* **30**, 6–7 (2002)

WEB SITE

Animation of mitotic recombination: http://www.nature.com/nrg/journal/v2/n8/animation/nrg0801_620a_swf_MEDIA1.html

TECHNOLOGY

A glowing fly trap

Drosophila researchers are notoriously resourceful when it comes to finding ways of extracting functional information from the fruitfly genome. By manipulating the transposable *P*-element, for instance, they have gained fast access to mutant phenotypes through insertional mutagenesis, and to gene expression profiles through enhancer trapping — in which a promoter-less reporter carried by a *P*-element reveals the expression pattern of an endogenous gene when 'captured' by the gene's enhancer. But these methods fail to inform about the behaviour of the protein expressed by the trapped gene — will it travel to the plasma membrane, rest on an organelle or degrade after a few minutes? Morin and colleagues have engineered a gene-trap vector that provides just such information: by tagging genes with a GFP molecule, they can identify the whereabouts of the encoded fusion product in living cells. Unlike antibody staining, which provides similar information, GFP protein tagging doesn't require any knowledge of the protein itself — it is quicker, can be done in living cells and, as the authors show, can bring to light (literally!) previously unknown genes.

The ability of the construct designed by Morin *et al.* to tag proteins requires flanking a GFP reporter gene with a splice acceptor and splice donor site, so that, when integrated into an intron, this foreign exon can be spliced between the amino- and carboxy-termini of a mature protein. The GFP construct lies within a *P*-element, by which it is delivered to flies and is mobilized to many random positions in the genome. In the more than 600 GFP-expressing lines of flies that the authors recovered, fluorescent proteins were collectively seen in virtually every cell compartment and, where known, their expression pattern faithfully recapitulated those of endogenous proteins, apparently without ill-effect on normal mRNA splicing or on protein folding. Curious to know which genes they had trapped in their screen, the authors sequenced the genomic DNA flanking 102 insertions; however, just under half did not match known or predicted genes — a sign perhaps that protein trapping can pick up unconventionally structured ORFs.

Although powerful, this technique does have its limitations. One intrinsic disadvantage is the insertion specificity of the *P*-element, which is biased towards genes with larger introns. Another one is the failure of the human eye to detect weak GFP signals — a drawback that an automated sorter can overcome. With more emphasis being given to understanding development in real time, the *in vivo* GFP protein markers created in this study will no doubt complement existing methods in flies for studying the dynamics of gene expression and cellular behaviour.

Tanita Casci

References and links

ORIGINAL RESEARCH PAPER Morin, X. *et al.* A protein trap strategy to detect GFP-tagged proteins expressed from their endogenous loci in *Drosophila*. *Proc. Natl Acad. Sci. USA* **98**, 15050–15055 (2001)

WEB SITE

William Chia's lab: http://www.kcl.ac.uk/depsta/biomedical/mrcdevbiol/Chia/Chia.htm