TECHNOLOGY

New delivery vehicles for Cre

As discussed in last month's special focus on mouse genomic technology (see below), new techniques are constantly emerging for switching on the site-specific recombinase Cre in spatially and temporally controlled ways. Now, two papers report new approaches to delivering the recombinase to undifferentiated and terminally differentiated cells — a self-deleting Cre-lentiviral vector that overcomes the cytotoxic effects of prolonged *cre* expression and a cell-permeable form of Cre. Because its uptake depends on protein trafficking, this cell-permeable Cre will probably both facilitate future gene-function studies and provide cell biologists with a useful new tool.

Traditionally, there have been two main ways to deliver Cre to target cells — by using *cre*-expressing transgenic mice or *cre*-carrying viral vectors. Although such vectors have their advantages, they predominantly infect dividing, undifferentiated cells. So to overcome this limitation, Alexander Pfeifer and colleagues created a new Cre-lentiviral delivery vector (LV-Cre) because lentiviruses can infect both dividing and non-dividing cells. When the authors injected LV-Cre into Rosa26–Cre (R26R) reporter mice, which ubiquitously express a Cre-activatable form of *lacZ*, β-gal staining was evident in both undifferentiated and terminally differentiated cells. However, when injected into the brains of R26R mice, this vector caused brain abnormalities to develop after several weeks. On closer inspection of Cre's effects, Pfeifer *et al.* found that its prolonged presence causes cell-cycle arrest and apoptosis in cells *in vitro* and *in vivo* — possibly because of Cre-mediated illegitimate recombination at 'pseudo' *loxP* sites in the mouse genome. So to prevent this cytotoxic activity, the authors developed a self-deleting form of LV-Cre (LV-Cre-SD), by inserting a single *loxP* site into the vector's 3′ UTR. When the vector is reverse-transcribed and inserted into the genome, this 3′ UTR region is duplicated and introduced into the 5′ UTR, creating a vector that is flanked with *loxP* sites. LV-Cre-SD mediates recombination both *in vitro* and *in vivo* at the same efficiency rates as LV-Cre but without its cytotoxic effects, proving that this self-regulating Cre vector is likely to be a useful new tool for activating or inactivating gene expression in dividing and differentiated cells.

Jo *et al.* took a different approach to delivering Cre to cells by developing a cell-permeable form of the protein that carries a membrane-trafficking sequence. When this protein is repeatedly injected intravenously or intraperitoneally into R26R mice over 3–5 days, it causes widespread *lacZ* expression throughout organs such as the brain, kidney and liver, without adversely affecting the mice. However, there are a couple of disadvantages to using this modified Cre — its widespread dissemination limits its use in tissue-specific studies and it mediates recombination in fewer cells than does Cre that is delivered by transgene expression. Nevertheless, this cell-permeable Cre is likely to become a useful tool for future gene-function studies, given its ease of use and systemic effects. Furthermore, because it provides a stable record of protein trafficking and uptake, it could provide a new approach to developing protein-based therapies for treating human disease and equip cell biologists with a handy new tool.

Jane Alfred

References and links

ORIGINAL RESEARCH PAPERS Pfeifer, A. *et al.* Delivery of the Cre recombinase by a self-deleting lentiviral vector: efficient gene targeting *in vivo*. *Proc. Natl Acad. Sci. USA* **98**, 11450–11455 (2001) | Jo, D. *et al.* Epigenetic regulation of gene structure and function with a cell-permeable Cre recombinase. *Nature Biotechnol.* **19**, 929–933 (2001)

WEB SITE

Special focus on mouse genome technology: http://www.nature.com/nrg/focus/mousegen/

IN BRIEF

VIRAL EVOLUTION

Recombination in the hemagglutinin gene of the 1918 "Spanish flu".

Gibbs, M. J. *et al. Science* **293**, 1842–1845 (2001)

A reanalysis of the gene sequence of the influenza virus that caused the 1918 pandemic — the 'Spanish flu' — has revealed that the major virulence determinant, encoded by the haemagglutinin (HA) locus, originated by recombination. Sequence alignment of three HA genes taken from the 1918 victims against modern-day isolates from humans, pigs and birds indicates that a recombination event occurred between swine- and human-lineage-derived HA sequences just before the 1918 pandemic, suggesting a causal link between the two events.

MOUSE GENOMICS

An SSLP marker-anchored BAC framework map of the mouse genome.

Cai, W.-W. *et al. Nature Genet.* **29**, 133–134 (2001)

A radiation hybrid transcript map of the mouse genome.

Avner, P. *et al. Nature Genet.* **29**, 194–200 (2001)

A radiation hybrid map of mouse genes.

Hudson, T. J. *et al. Nature Genet.* **29**, 201–205 (2001)

These papers report new resources for the sequencing and functional analysis of the mouse genome. The first reports an SSLP-marker-anchored BAC map of the mouse genome that was generated by using specific probes for library screenings, selected by improved oligo-designer software. This map covers 94% of the genome in 600 contigs. Avner *et al.* constructed a radiation-hybrid (RH) transcript map of 5,904 mapped EST and STS markers. The mapped ESTs were isolated from a mouse embryonic endoderm library to enrich for transcripts expressed in early development and unlikely to have been previously mapped in humans. Together with the RH map reported by Hudson *et al.* — which contains 11,109 genes positioned relative to a map of 2,280 markers — these maps provide resources for sequencing the mouse genome, for orthology mapping in humans and for rapidly identifying genes mutated in ENU mutagenesis screens.

FUNCTIONAL GENOMICS

Large-scale identification of mammalian proteins localized to nuclear sub-compartments.

Sutherland, H. G. E. *et al. Hum. Mol. Genet.* **10**, 1995–2011 (2001)

Incorrect nuclear protein localization has been associated with human disease and cancer. Sutherland *et al.* tracked subnuclear protein localization in mouse embryonic stem cells using a previously developed gene-trap screen, in which a *lacZ* marker is inserted into introns and the chimeric proteins are detected by immunostaining. The authors found that proteins that share subnuclear localization contain similar domains, indicating that the localization of these proteins can be predicted from their sequence.

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