

ANALYSIS

and colleagues, are important elements to add to the lab-on-a-chip tool kit. Their work represents the first demonstration of a micro-fabricated fluidic device for cell sorting that is based on a fluorescence signature. Instead of breaking the fluid stream into droplets and separating the cells by electrostatic deflection, this device electrokinetically directs cells from an analysis channel into one of two possible output channels by simply selecting which output channel is connected to the driving voltage. Using this system, the workers were able to achieve enrichments of 30- to 100-fold depending on the experimental details, and for cells, 20% viability after sorting. The throughput was lower than conventional fluorescence-activated cell sorting (10–20 Hz), but the sorting speed is likely to be improved by using higher electric field strengths and parallel sorting structures.

In the device described in this study, the cells were confined laterally by the use of narrow channels so that they were forced to transit through the probing laser beam. However in another variation of a miniature flow cytometer reported last month in *Analytical Chemistry*, electrokinetic focusing (analogous to hydrodynamic focusing used in conventional cell sorters) was used for spatial confinement, allowing the use of broader channels⁵.

The ultimate goal of microfluidics is to integrate as many different chemical processes as necessary to solve a given measurement problem on a single device. On this front, there have been some successes with low levels of integration, including mixing of reagents to induce a chemical reaction, followed by chemical separations^{6,7}. Further degrees of integration will likely be demonstrated in the near future.

One of the visions of developers of lab-on-a-chip devices is to bring the plug & play versatility of a video game to laboratory experimentation. Like a video game, you will have a generic controller that accepts microfluidic chips rather than game cartridges. Microfluidics chips would be designed to perform different assays, but all would be accepted and operated by the generic controller. The result could be a versatile and powerful research tool. In fact this goal is now a commercial reality in applications focusing on chemical separations⁸. More complex applications will surely be addressed by such plug & play strategies as higher degrees of microfluidic integration are demonstrated.

Lab-on-a-chip devices have progressed rapidly, but there are remaining obstacles that must be overcome to achieve the full power that many envision. One area required for high-throughput processing and analysis that needs improvement is the ability to bring samples to devices in rapid succession; we call this the “world-to-chip” interface. Other areas that will become quite valuable

are fabrication processes that will allow viable manufacturing of three-dimensional fluidics devices and spatially heterogeneous patterning of surface chemistries, and sensitive detection systems in addition to optical fluorescence and mass spectrometry. Finally, computer-aided design tools that include fluidics, chemistry, and biochemistry will be necessary to rapidly design the future generations of fluidics microchips, such as micro-electronics chips are designed today.

The future for microfabricated fluidics devices—or the lab-on-a-chip—looks quite promising. We can only imagine what type of microfluidic systems will emerge in the coming

decades to replace the question mark in Figure 1, but they are sure to intrigue, and indeed, may transform the way we conduct research.

1. Fu, A.Y. et al. *Nat. Biotechnol.* **17**, 1109–1111 (1999).
2. Jacobson, S.C., Culbertson, C.T., Daler, J.E. & Ramsey, J.M. *Anal. Chem.* **70**, 3476–3480 (1998).
3. Ermakov, S.V., Jacobson, S.C. & Ramsey, J.M. *Anal. Chem.* **70**, 4494–4504 (1998).
4. He, B. & Regnier, F. *J. Pharm. Biomed. Anal.* **17**, 925–932 (1998).
5. Schrum, D.P., Culbertson, C.T., Jacobson, S.C. & Ramsey, J.M. *Anal. Chem.* **71**, 4173–4177 (1999).
6. Jacobson, S.C., Hergenroder, R., Moore, A.W. Jr. & Ramsey, J.M. *Anal. Chem.* **66**, 4127–4132 (1994).
7. Chiem, N.H. & Harrison, D.J. *Clin. Chem.* **44**, 591–598 (1998).
8. Hewlett-Packard HP 2100 Bioanalyzer. <http://chem.external.hp.com/scripts/>

The right time and place for molecular scissors

Lothar Hennighausen and Priscilla A. Furth

The development of genetic tools has been and will continue to be a driving force in biological discovery. In particular, the ability to mutate genes within the mouse genome has been instrumental in the identification and understanding of genetic pathways controlling organogenesis and tumorigenesis. Now, Wen-Hwa Lee and colleagues¹ report experiments that take the application of molecular tools to new heights. They combined the generic *Cre-loxP* recombination system with a tetracycline-dependent switch and topped it off with tissue-specific control elements. This technical voyage will enable biologists to precisely manipulate individual genes in specific tissues and at predetermined time points.

What are the fundamentals of advanced gene switches? During the past decade engineers in many molecular workshops around the world have searched through the tool boxes of prokaryotes and turned up gadgets and gizmos that greatly aided molecular engineers in manipulating the mouse genome with an ever-increasing sophistication. Included in our repertoire are the tools of

homologous recombination that were developed by the groups of Smithies² and Capecchi³, the *Cre-loxP* recombination system developed by Sternberg⁴ (originally isolated from the bacteriophage P1), and the tetracycline control switch developed by Bujard⁵ (composed of components of the bacterial tetracycline repressor and the herpesvirus transcription element VP16). Lastly, DNA control elements that have the capacity to direct gene expression to specific cell types have been identified over the past decade.

Why do we need sophisticated gene switches? The mouse is the organism of choice to study mammalian development, physiology, and cancer. Analyses of genetic pathways in the mouse, through the deletion of individual genes by conventional homologous recombination, is powerful but can run into roadblocks. Researchers who wish to study a particular gene in a specific organ often encounter problems. First, the deletion of a gene from every cell in a mouse (the standard knockout technique) can result in embryonic lethality, and thus the tissue is not available for analysis. For example, mutations in the *BRCA1* gene predispose women to breast cancer, but its analysis in mouse mammary tissue was accomplished only recently through a tissue-specific gene deletion⁶. Second, even if the deletion of a gene by conventional homologous recombination leads to the expected phenotype, it still needs to be determined whether the defect is cell autonomous or the result of systemic or cell–cell interactions.

Individually, cell-specific *Cre-loxP* mediated recombination⁵ and a cell-specific tetracycline-dependent time switch^{7,8} have been used successfully in mice. Early efforts to fuse

Lothar Hennighausen is chief of the laboratory of Genetics and Physiology, National Institute of Diabetes, Digestive and Kidney Diseases, National Institutes of Health, Bldg. 8, Rm. 101, Bethesda, MD 20892 (mammary@nih.gov) or (<http://mammary.nih.gov/lgp>). Priscilla A. Furth is associate professor of medicine and physiology at the Institute of Human Virology, departments of medicine and physiology, University of Maryland Medical School, 725 West Lombard Street, Room 545, Baltimore, MD 21201-1192 (furth@umbi.umd.edu).

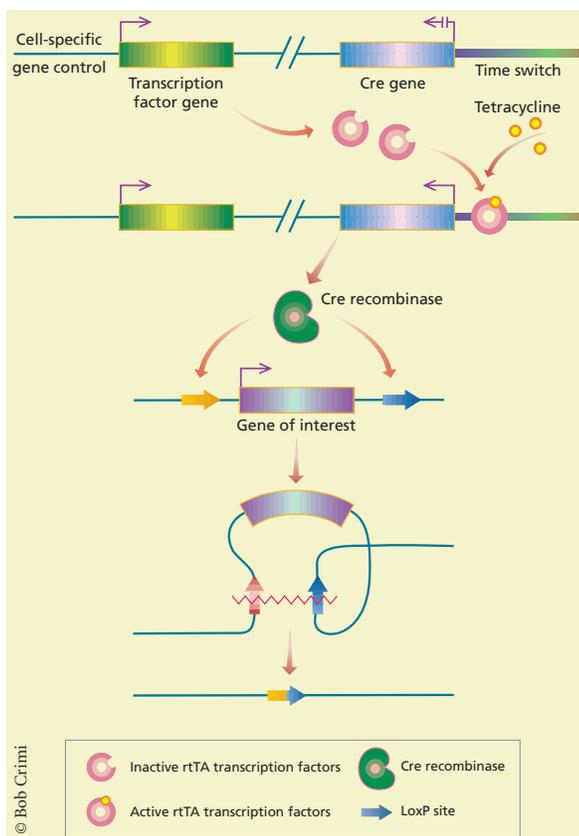


Figure 1. Strategy for the deletion of genes from specific cell types at defined time points. The transcription factor gene encodes the tet-VP16 hybrid protein (rtTA) and is under control of a cell-specific promoter, such as the mammary-specific promoter from the whey acidic protein (WAP) gene and the Rb gene promoter. The gene encoding Cre recombinase (*Cre* gene) is under control of the tetracycline operator (time switch). The rtTA transcription factor is produced in cells targeted by the WAP and Rb promoters. However, the rtTA transcription factor is inactive in the absence of doxycycline, since it cannot bind to the tetracycline operator (time switch) by itself. Doxycycline binds to the rtTA transcription factor, inducing a conformational change and resulting in the interaction of rtTA with the tetracycline operator. The binding of the rtTA to the time switch supports the establishment of a transcription complex and the activation of the Cre recombinase gene (*Cre* gene). Cre recombinase is produced and can delete a gene of interest that has been flanked by loxP sites. The outcome of this event is a chromosomal locus that is devoid of the gene of interest and contains one loxP site as a reminder of the former gene.

a tetracycline-dependent time switch to the Cre recombinase demonstrated its feasibility⁹. Before the development of the tet-on system by the Bujard lab¹⁰, St-Onge and colleagues tested the combination of the Cre-loxP system with a tet-off switch⁹. These investigators used a promoter from the human cytomegalovirus (hCMV) that targeted expression of the tetracycline control switch (tTA) to a wide variety of tissues. Cre-dependent homologous recombination was observed after the withdrawal of tetracycline⁹.

How does the study by Lee and coworkers advance our ability to study genetic pathways in the mouse? These investigators succeeded in targeting temporal controlled gene^{11,12} deletions to two important tissues:

neural and mammary. They linked mammary-specific control elements from the WAP (whey acidic protein) gene¹² to the rtTA transcription factor gene from the tet-on system¹⁰ and thus targeted its expression to mammary epithelial cells. They linked a Rb gene promoter to rtTA and obtained neural specific activation. The WAP and Rb mice also carried the Cre recombinase gene under control of a promoter containing tetracycline operators (Figure 1). These operator units did not bind to the rtTA transcription factor unless doxycycline, a tetracycline derivative, was present. Upon binding of rtTA to the tetracycline operators, a transcription complex was assembled on the promoter and the *Cre* gene was transcribed. Upon removal of doxycycline the transcription complex disintegrated and the *Cre* gene was inactivated. Thus doxycycline administration triggered the time switch. When the mouse was supplied with doxycycline in the drinking water the switch was on, and it was turned off upon removal of the antibiotic. The use of doxycycline and the tet-on switch to activate Cre expression appears to give the investigator tighter temporal control than withdrawal of tetracycline does in the original tet-off system.

Where will this study take us? Tools that enable us to directly control expression and loss of expression of

tumor suppressor genes, oncogenes, and other growth factors at different stages of cancer progression will help us determine their specific roles at different times. Gain-of-function experiments have provided compelling evidence that gene function during hyperplasia can be separated from its action during metastatic disease. Already we know that the continued presence of an oncoprotein is not always required to maintain malignant progression⁷.

Importantly, the study by Lee and coworkers immediately opens the door for new and exciting investigations in both mammary and neural tissues. For example, researchers will now be able to delete candidate tumor-promoting genes at specified stages during breast

cancer progression in mouse models.

On a more futuristic note, we can look forward to the day when deletion of a specific gene can be accompanied by tetracycline-dependent conditional activation of the same or a different gene. For example, the rtTA that activates the *Cre* transgene in the mice developed by Lee and co-workers could be used to simultaneously activate tetracycline-dependent expression of another transgene. Such experiments could be used to test for compensation and synergy of specific genetic changes.

Although the present study represents a major step forward, the “temporal tissue scissors” are far from perfect and are not yet an “off-the-shelf product” (and may never be). Several problems need to be solved. Most pressing is the mosaic expression of the transgenes. Only about 12% of the epithelial cells undergo Cre-mediated recombination. Such mosaicism has been observed with other transgenes and may reflect a general feature of some transgenes and/or genomic integration sites. For some experiments, this degree of heterogeneity may be acceptable and even exploited, as the cells exhibiting recombination are surrounded by their own nonrecombined control cells. However, for other experiments, a higher frequency of recombination will be required to distinguish effects of the gene deletion in experimental recombined cells from the nonrecombined control cells.

Another problem was that not all of the transgenic lines expressed the *Cre* gene. This problem may be overcome by using homologous recombination to target the *Cre* gene to endogenous loci, rather than using transgenes. Last, there will always be some illicit recombination in the absence of tetracycline. Investigators who wish to target new tissues may need to generate and test multiple lines of mice before they find the optimal tool.

Despite these limitations, this advance should inspire investigators and funding agencies alike to push the development of conditional tools for mouse genetics. These tools will be needed to generate mouse models that more accurately reflect the course of human disease.

1. Utomo, A.R.H., Nikitin, A.Y. & Lee, W.-H. *Nat. Biotechnol.* **17**, 1091–1096 (1999).
2. Doetschman, T. et al. *Nature* **330**, 576–578 (1987).
3. Thomas, K.R., Folger, K.R. & Capecchi, M.R. *Cell* **44**, 419–428.
4. Sternberg, N. & Hamilton, D. *J Mol Biol* **150**, 467–486 (1981).
5. Gossen, M. & Bujard, H. *Proc. Natl. Acad. Sci. USA* **89**, 5547–5551 (1992).
6. Xu, X. et al. *Nature Genetics* **22**, 37–43 (1999).
7. Ewald, D. et al. *Science* **273**, 1384–1386 (1996).
8. Furth, P.A. et al. *Proc. Natl. Acad. Sci. U.S.A.* **91**, 9302–9306 (1994).
9. St-Onge, L., Furth, P.A. & Gruss, P. *Nucleic Acids Res* **24**, 3875–3877 (1996).
10. Gossen, M. et al. *Science* **268**, 1766–1769 (1995).
11. Gordon, K. et al. *Bio/technology* **5**, 1183–1187 (1987).
12. Wagner, K.-U. et al. *Nucleic Acids Res.* **25**, 4323–4324 (1987).