Laser-Controlled Cells

SIGHTINGS

Scientists studying development want to control the timing of gene expression in cells and animals. In the past, researchers have used tissue-specific promoters to target genes to a variety of cell types. Gene expression has also been put under the control of small molecules that can diffuse into cells and turn an engineered promoter on or off. However, until recently (*1*), none of these systems has allowed precise regulation of a single cell at any selected moment in time.

Heat shock proteins are ubiquitous, found in organisms from humans to yeast. Their promoter elements respond to elevated temperature by producing more RNA transcripts. By replacing the endogenous heat shock gene with another gene, the heat shock promoter becomes a useful laboratory tool with which virtually any gene can be regulated by heat.

Halfon *et al.* (*1*) show that by raising the temperature of the heat shock protein promoter (hsp) with a laser, gene expression can be regulated in single cells in developing embryos of the fruit fly *Drosophila*. Similar experiments have been performed with the worm *Caenorhabditis elegans* (*2*, *3*).

For the fruit fly studies, the researchers used transgenic embryos carrying the hsp26 promoter that drives expression of the reporter gene lacZ. When whole flies are heat shocked, they turn blue when they are stained for LacZ. To visualize single target cells, Halfon *et al.* configured a microscope with digitally enhanced differential interference contrast optics. The resolution was sufficient to detect every cell in the developing embryo.

Next, the researchers set up a pulsed dye laser that delivers a beam with a focused spot 1 μm × 2 μm in size. The output from this laser is powerful enough to kill cells, and, in fact, it had been used for ablation studies in the past. The trick in the heat shock experiments was to develop a series of filters to reduce the laser energy so that the target warmed rapidly to only around 35°C, sufficient to trigger the promoter but not enough to injure the cells. The timing of the pulses was also varied from 1 to 2 min.

Through trial and error, Halfon *et al.* were able to regulate LacZ expression in two cell types, neurons and muscle cells. Under optimum conditions, 50% of all cells exposed to the laser beam responded. The precise nature of the targeting was impressive; they never saw more than the one selected cell turn blue.

By regulating the expression of DNA recombination enzymes (such as FLP or cre), all progeny from any heat-targeted cell should be able to be permanently marked. If the recombination product yields a functional reporter gene (such as green fluorescent protein or LacZ) all progeny can easily be identified in situ. A twist to this approach would be to use targeted recombination to actually delete an entire gene from the genome. Whole-animal studies may also become possible with the use of fiber optic microcatheters to precisely deliver laser energy in vivo.

–Robert Sikorski and Richard Peters

References

1. M. S. Halfon, H. Kose, A. Chiba, H. Keshishian, *Proc. Natl. Acad. Sci. U.S.A.* **94**, 6255 (1997).
2. E. G. Stringham and E. P. M. Candido, *J. Exp. Zool.* **266**, 227 (1993).
3. J. Harris, L. Honigberg, N. Robinson, C. Kenyon, *Development* **122**, 3117 (1996).

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Selecting the Needle

SIGHTINGS

Hardly a week passes without the discovery of a new human or mouse gene. The technology usually involves the generation and manipulation of big pieces of DNA. The materials and methods section in any of these reports reveals routine studies of DNA in the 100- to 1000- kilobase range. Moving along the genome in these great overlapping leaps is critical to the success of the Human Genome Project.

At best, today's methods for making libraries of large genomic fragments in both yeast and bacteria are challenging. An investigator wishing to clone a fragment of the human genome containing all of a particular gene would have to undertake the laborious task of screening through a library and then carry out extensive mapping studies. This is analogous to looking for the proverbial needle in a haystack. In addition, overlapping clones may need to be assembled into a larger unit (a "contig") to produce the desired clone. Rather than screening for the needle, wouldn't it be better to "select" for it?

A recent report by Larionov *et al.* (*1*) shows how. Their technique, called TAR for transformation-associated recombination, rapidly selects clones containing any desired genomic fragment in yeast. The key to the TAR approach is the remarkable specificity of homologous recombination in the yeast *Saccharomyces cerevisiae*. The art of transformation using yeast is well honed, and complex mixtures of DNA can be routinely introduced into yeast at a high rate. Clones can be selected for viability based on the fact that all DNA in yeast must exist in a circular form or have telomeric ends. Therefore, only when recombination produces a circle or a linear molecule with telomeres will it propagate to the next generation. The linear telomere approach has been used in the past to produce functional adenovirus clones in yeast (*2*), a pioneering study that provided the conceptual foundation for the development of TAR.

For their TAR test case, Larionov *et al.* chose to isolate for the first time the genomic locus containing all of the gene BRCA2. They started by creating a circular plasmid intermediate and then linearized it to produce a DNA fragment with the following structure: BRCA2 promoter (669 base pairs)–centromere–HIS3–BRCA2 last exon (308 base pairs). They then added this DNA fragment to total human genomic DNA and transformed the whole mix into his3-yeast, selecting for HIS3+ cells. Intracellular recombination between the ends of the cloned sequence (promoter and last exon) and the human DNA produced a circular form that contains all of the DNA from promoter to last exon—namely, the entire BRCA2 gene. The direction that the promoter and last exon were cloned into the first plasmid is important [see figure 1 of (*1*)].

They obtained about 1000 HIS3+ clones and pooled them into 33 pools and screened these by polymerase chain reaction for BRCA2 sequences. This step was necessary because of the high numbers of background clones. In the end, they obtained three positive pools and went on to identify the exact BRCA2-containing yeast. Through a variety of analyses, they showed that the final DNA product from recombination was indeed exactly as predicted.

The TAR approach will likely have wide application in genetics labs. Those attempting to produce genetically altered mice should look at the TAR method. With a mouse clone in yeast, the gene can be easily manipulated through in vivo recombination, avoiding the time-consuming steps now done in vitro.

–Robert Sikorski and Richard Peters

References

1. V. Larionov, N. Kouprina, G. Solomon, J. G. Barrett, M. A. Resnick, *Proc. Natl. Acad. Sci. U.S.A.* **94**, 7384 (1997).
2. G. Ketner, F. Spencer, S Tugendreich, C. Connelly, P. Heiter, *ibid.* **91**, 6186 (1994).

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