Primary Target

SIGHTINGS

Scientists who work on systems that allow genetic manipulation seem to get all the breaks. Those who study organisms such as *Drosophila* or yeast seem to be limited less by technology than by their creative imagination. Genes can be knocked out, tagged, or mutated at will. Not so, however, for tissue-culture systems, where techniques are more confining.

There are certain cell lines from chickens (*1*) or mouse embryos (*2*) that do undergo homologous recombination at relatively high frequencies, and thus allow facile gene targeting. But what about the thousands of cell lines and primary cells from an animal? Targeting primary cells also presents the problem that these cells divide for a fixed number of times in culture. Techniques for targeting and cloning out the correct isolates must, therefore, be efficient.

A group led by John Sedivy at Brown University has been working on the problem of targeting cell lines for some time. In the 8 August 1997 issue of *Science* (*3*), they report the successful creation of a nonimmortalized cell strain with a homozygous deletion in a critical protein, p21. They succeeded by a combination of trial and error, and attention to detail.

It is well known that "older" cells divide fewer times when put under culture conditions. Human foreskin fibroblasts divide about 70 to 80 times in culture before they senesce, whereas cells from fetal tissue can multiply as much as 100 times. The Sedivy group intelligently started with a primary cell that would double the most times in culture, fetal human lung fibroblasts.

Next, they used two targeting vectors to replace both copies of the p21 gene with mutant versions. Key to this step was the use of targeting vectors that "trapped" the p21 gene promoter. Only correctly recombined cells would express the drug resistance markers that were introduced on the targeting constructs. This approach has a higher efficiency of targeting than the positive-negative selection schemes commonly used for creating mouse knockouts in ES cells. This method is therefore necessary when targeting somatic cell lines or cell trains.

Finally, the group had to find culture conditions that would allow efficient cloning during their various manipulations. They found that certain media and serum concentrations would allow single-cell cloning, that is, allow single cells to grow into a population of identical clones. This was critical, because many primary human cells will not even grow at low density.

In the end, they succeeded in deleting both copies of p21 and uncovering the fact that the gene is actually required for senescence itself. There were no fancy twists here, just hard work, carefully planned experiments, and persistence. Mouse researchers will look at the paper and wonder what all the fuss is about. After all, gene targeting has been done in the rodent for some time now. However, most scientists in the world do not work with mice. Mice are expensive to use, and they may not be the best system for studying every gene. Cell lines are universal tools; a method to manipulate their genes will no doubt have broad application.

–Robert Sikorski and Richard Peters

References

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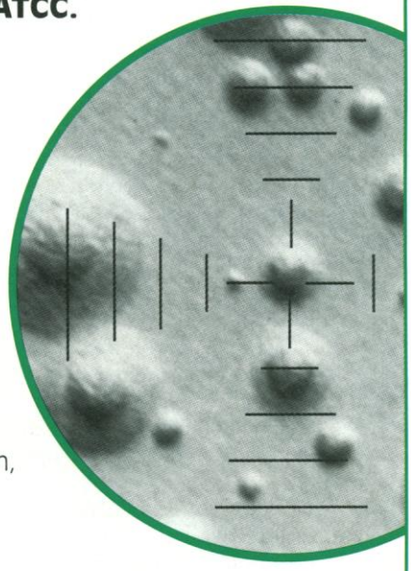

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