ASM CONFERENCE ON INDUSTRIAL MICROORGANISMS

DOUBLE WHAMMY FOR LOCKING GENES IN PLACE

BLOOMINGTON, Ind.—Sandwiching a gene of interest between two amplifiable genes that serve as dominant selectable markers acts as a "double whammy" to lock the construct in place, according to Rodney Kellems of Baylor College of Medicine (Houston, TX). Kellems, speaking here at October's Fourth American Society for Microbiology Conference on the Genetics and Molecular Biology of Industrial Organisms, sees the strategy as "very attractive from a commercial standpoint"—it has already proved a workable alternative for producing tissue plasminogen activator (t-PA) on a small scale.

The first selectable marker gene produces adenosine deaminase (ADA). Kellems and his colleagues had already developed a culture medium for selecting cells that overproduce ADA. After repeated selection cycles, the research group isolated variant mouse cells that contain 10,000-fold more ADA activity than usual, an increase that corresponds very closely to specific amplification of the ADA gene. The amplified gene may account for more than five percent of the cell's genomic DNA under some conditions, and the ADA protein for more than three-fourths of the cell's soluble protein.

Such genetic constructions, which slow cellular growth rates, tend to be unstable—particularly if the selective pressure is removed. Moreover, when other genes of interest are associated with the amplified ADA gene, their maintenance in cells depends solely on physical linkage to ADA—a reliance that is less than ideal because "co-amplification is not so faithful," Kellems explains. "You have to keep an eye on it."

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Fluorescent photomicrograph of ethidium-bromide-stained chromosomes from a mouse cell line that has a 5,000-fold amplification of the selectable ADA gene. When gene amplification occurs in mouse cells, the excess genetic material typically appears in "double minute" chromosomes (the dot-like structures surrounding all the chromosomes).

Thus, to assure greater stability of valuable genetic constructs, Kellems and his collaborators recently turned to a double-selection approach, by adding a dihydrofolate reductase (DHFR) marker to the construct. Maintaining the selectable genes—and others that lie between them—involves subjecting the cells to both selective pressures simultaneously, or by alternating between the two, Kellems notes. The selection protocols for the ADA and DHFR markers "are compatible," so that the "stability of such constructs" is assured.

—**Jeffrey L. Fox**

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GENETIC OPTIONS FOR EXPLOITING YEAST

Bloomington, Ind.—The *Ty* retrotransposon in yeast is a genetic element that can hop about the yeast genome and, after appropriate engineering, spew off large quantities of virus-like particles in which useful proteins may be sequestered. Apparently a crippled retrovirus, the engineered element may prove useful for producing vaccines, including one containing proteins from the human immunodeficiency virus (HIV) to protect against AIDS, says Alan Kingsman of Oxford University (Oxford, U.K.).

Speaking at October's Fourth American Society for Microbiology Conference on the Genetics and Molecular Biology of Industrial Organisms, Kingsman explained that the *Ty* genetic element, which is "a few kilobases in length," produces an RNA molecule that can be transcribed to produce several proteins. These proteins tend to aggregate about the RNA, assembling into virus-like particles about 60 nM in diameter that resemble a "retrovirus without an envelope." Ordinarily this structure seems to help move the element from one site in the yeast genome to another.

To exploit the retro-transposon's natural productivity, Kingsman and his collaborators have incorporated into it new genes that specify proteins from other organisms. For example, they have added the gene from HIV that specifies the p24 protein. Under such circumstances, the yeast cells make a virus-like particle that contains "hybrid proteins"—part HIV- and part *Ty*-specified. The uniformly sized particles then can be "very easily purified," Kingsman says. Some of the incorporated HIV p24 protein is studded on the outside of the particles making them "highly immunogenic" when injected into rabbits and rats, in which they elicit anti-p24 antibodies.

"We have a powerful procedure for making reagent antibodies," Kingsman says. Once optimized, he concludes, the process may prove useful as a "generic technology for vaccine development." —**JLF**