ACS MEETING

SOLVING THE SHORTAGE OF HUMAN CELL LINES

NEW ORLEANS— Mammalian cells — especially human cells — are complicated entities that we understand imperfectly. Yet, biotechnologists often find these cells to be the only appropriate sources from which to isolate therapeutic compounds. And for expressing genetically engineered human proteins that require absolute fidelity of post-translational modification, human cell lines are probably the sole choice.

DIAGRAM BY F. LINDON; COURTESY R. KAUFMAN, GENETICS INSTITUTE

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Synthesis and processing of factor VIII. The primary translation product is translocated to the endoplasmic reticulum (RER), where most of it is bound to a resident protein (BiP) and then degraded. Some of the product folds in a manner that is compatible with its transport to the Golgi, where post-translational modifications—such as adding metal groups (Me), sulfates, and sugars—occur. Next, the molecule is cleaved to form heavy and light chains, which are then secreted into the medium. If von Willebrand factor (vWF) is present, a stable complex is formed; otherwise, the chains are degraded.

Unfortunately, there is a real paucity of such cell lines. The reason, as Charles F. Goochee (University of Houston, TX) pointed out at the American Chemical Society meeting in early September, is that normal, primary human cells have a limited life span in culture. Even tumor cells are rarely immortal. Unlike their rodent counterparts, human cells almost never undergo spontaneous immortalization. And, added Goochee, current techniques for immortalizing cells—somatic cell mutagenesis or chemical mutagenesis—have not proved widely applicable to human systems.

What does seem to work, at least to some extent, is exploiting the ability of oncogenes to affect the growth properties of cells—including extended life span and reduced growth factor requirements—during neoplastic transformation. Goochee and coworkers have concentrated on introducing SV40 large T (a nuclear oncogene isolated from a DNA tumor virus) into human embryonic kidney (HEK) cells by transfection. Normally, these cells have a very short life span in culture, about 15–20 generations. The transfected cells, however, reached 100 generations before they senesced. Transfected cells, which are morphologically indistinguishable from primary cells, constitutively synthesize and express the oncogene. They grow at the normal rate, ceasing at confluence, and they produce single-chain urokinase at levels comparable to those found in primary kidney cells. Although they are not truly immortal, concluded Goochee, "If we could push the life span to 150 generations in serum-free medium, then someone could conceivably think of producing a recombinant product in those cells."

Given the difficulties of creating established human cell lines, it is fortunate that not all human proteins need "perfect" post-translational modification to function properly. Proteins such as erythropoietin and factor VIII, for instance, are currently being produced in Chinese hamster ovary (CHO) cells. Randal J. Kaufman's group at Genetics Institute (Cambridge, MA) has used a dihydrofolate reductase (DHFR) selectable marker system to introduce and co-amplify foreign genes in dihydrofolate-deficient CHO cells. By selecting for resistance to increasing levels of the drug methotrexate, it is possible to amplify both genes.

The Genetics Institute researchers found it relatively straightforward to introduce the factor VIII gene into CHO cells and achieve expression—but only in the presence of 10-percent serum. Without it, the molecule's heavy and light chains that appear in the medium are degraded. The magic ingredient in serum turns out to be von Willebrand factor.

The way to produce factor VIII in serum-free medium, then, is to introduce the gene for von Willebrand factor (with a different amplifiable marker) into the factor VIII-expressing system. And, in fact, Kaufman says that co-expressing cells accumulate factor VIII linearly with time.

Even though factor VIII does not require "perfect" post-translational modification, it goes through extensive processing within the CHO cell. According to Kaufman, most of the primary translation product is never transported out of the endoplasmic reticulum; it is merely degraded. Some of the product folds in a manner that is compatible with its transport to the Golgi apparatus. There, most of the N-linked glycosylation sites are modified to complex with sugars. O-linked sites are added, as well as sulfate and tyrosine residues. Finally, says Kaufman, the molecule is cleaved to generate the heavy and light chains. These are secreted into the medium, where they are degraded if von Willebrand factor is not present.

An elegant solution, but would the path through a human cell be any less tortuous? **—Jennifer Van Brunt**