EXPRESSION SYSTEMS

PHILOM BIOS MAKING BIG PLANS FOR MINICELLS

SASKATOON, Sask.—While northwestern Canada is not considered exactly a hotbed of technological innovation, the founders of Philom Bios are seeking to change that.

Incorporated at the end of 1980, the company expects to have raised some $1.5 million by the end of February through the private placement of 300,000 shares at $5 each. What projects does the four-employee start-up (which currently lacks in-house research and laboratory space) have in the works to attract such investment? The main goal is to commercialize anucleated minicells as vehicles for vaccines and for the production of valuable biologicals. Philom Bios also intends to develop microbes to produce alcohol efficiently, to work on deriving useful compounds from plants, and to perform contract research.

First observed back in the 1920s, minicells are formed when a bacterium divides unequally, resulting in one standard daughter cell containing a nucleus, and one small, anucleated cell dubbed the minicell. Because they have no genetic material of their own, minicells have long been a valuable research tool—scientists can insert a plasmid into a minicell to monitor the plasmid's function.

Philom Bios hopes to capitalize on work by George G. Khachatourians of the University of Saskatchewan. Khachatourians, principal scientific advisor to the company, has U.S. and Canadian patents on an *Escherischia coli* minicell vaccine against infectious diarrhea in calves. Because minicells don't replicate, administering a pure preparation should carry no risk of infection. The idea is to insert a plasmid coding for the pathogen's surface antigen into a minicell-producing strain of *E. coli*. Minicells derived from this cell line will produce the desired surface antigen and might thereby provide a safe, "live" vaccine. The company estimates a potential $40-million Canadian market and $375-million U.S. market for its calf scours vaccine.

According to John V. Cross, president of Philom Bios, Khachatourians has developed a proprietary method for inducing the production of a minicell with virtually every cell division. "For every 10^9 viable cells in a culture, at least 10^9 anucleated cells (minicells) are produced," states Khachatourians' U.S. patent, granted two years ago.

The scientist can now purify the minicells to the point where for every 100 million non-reproducing cells there will be only one whole cell. These results are some two or three orders of magnitude better than other laboratories report. One step of the purification process involves culturing the cells in the presence of an antibiotic that kills only growing cells (those that have nuclei); the other procedures are proprietary.

"It's not enough, though, is it?" points out John N. Reeve of the microbiology department at Ohio State University (Columbus). "You never get rid of all the nucleated cells. How many live, pathogenic organisms do you want to vaccinate someone with?" Reeve says the idea might work for some low-infectivity diseases in which a large number of bacterial cells are needed to cause an infection, but the technique could be dangerous for high-infectivity diseases.

Cross agrees, noting that the company will target diseases where the introduction of a very small number of whole cells would not cause an infection. He also says Philom Bios hopes to boost the purity of its preparation another order of magnitude or more. According to Cross, on the order of 10^6 minicells are needed for each vaccine dose—down from the 10^{12}–10^{13} stated in Khachatourians' original patent. This comes out to only about 1 ml of fermentation volume per dose, he says.

Reeve maintains that using minicells as vaccines is not a new idea: "I talked with George Khachatourians about it 10 years ago." While he acknowledges that minicells can raise the proper anti-sera to elicit protection, he questions if they are any more effective as vaccines than heavily UV-irradiated normal cultures.

Roy Curtiss of the department of biology at Washington University (St. Louis) has developed minicell-producing strains of *Salmonella typhinurium*, the most common *Salmonella* food poisoning agent and the cause of typhoid in mice. Killed *Salmonella* vaccines are not usually very effective, he says, but he found that the minicells did not work any better.

Cross counters: "We have the suggestion that the antigenicity concentration can be higher in minicell applications." He cautions, however, that minicells do not represent a blanket improvement.

Philom Bios' other planned application of minicells is as a production vehicle. "If you think of the minicell as a little biological factory, you'll appreciate that it has a much longer production life than a whole cell that goes through regular life cycles," says Cross. He envisages a production sys-

Electron micrograph of an *E. coli* cell dividing to produce a minicell.

PHOTO COURTESY JOHN REEVE OHIO STATE UNIVERSITY

HP GENENCHEM

CAUCCUUGAGGUGAAAAUGAGAACUGUCACGAAAUG

If you thought the base sequence under HP Genenchem in the company's logo (above) encoded a revolutionary peptide that the firm intends to market, allow us to clarify the issue. In today's world of intricate, elaborate, and sometimes stunning corporate logos, this company has opted for a simpler-looking but nonetheless elegant scheme. The sequence of nucleic acid bases spells out HP Genenchem using the single-letter abbreviations for the amino acids that would be coded for: histidine (**H**), proline (**P**), topaz (stop codon), glycine (**G**), glutamic acid (**E**), asparagine (**N**), glutamic acid (**E**), asparagine (**N**), cysteine (**C**), histidine (**H**), glutamic acid (**E**), methionine (**M**).

Interestingly, the company uses synonym codons whenever possible (for example, using CAU for histidine in the first case and CAC in the second). Also, the choice of UGA (topaz) as the stop codon is not without thought. The stop codons UAA (ochre) or UAG (amber) would have represented more definitive breaks, but the designers of the logo opted for the weakest stop codon to represent the half-space in the logo.

If the staff of HP Genenchem (South San Francisco, CA)—a year-old joint venture between Genentech and Hewlett-Packard—is as clever in developing biotech instrumentation as it was in designing its logo, then rivals may have reason to worry.

—GAGGACAGUUAA