- monoclonal antibodies for diagnostic reagents (veterinary use)
- proteases and enzyme engineering

The Institut Pasteur will concentrate on:

- medicine: vaccine production through genetic engineering, dosages, and diagnostics, vector production, and neurobiology
- microbiology: gene cloning in *Bacillus subtilis*
- bioinsecticides
- methylotrophic bacteria
- chemical technology applied to genetic engineering (peptide synthesis and oligonucleotides)

Additional contracts will be awarded in areas not being covered by the main research institutions. Private or public teams will be awarded these "priority contracts," which will deal with such topics as enzymes (amylases), nitrogen fixation, fermentation, and improvement of wine. Foreign contracts will include several projects with the EEC (mainly concerning data centers for nucleic acids and enzymes), as well as agreements with Canada, Japan, and possibly Norway, Sweden, and Austria.

Microbiology is one area in which the program will concentrate, with emphasis upon industrial microbiology. This field is almost completely ignored in France, necessitating the active recruitment of foreign technicians. Fermentation techniques make up another targeted area in which France is presently weak compared to West Germany, Japan, the United States, and the United Kingdom. The research aims of scientists working on enzyme engineering will be modified for industrial application. The routine search for new strains, the preparation of hyperproductive strains by mutagenesis, and genetic engineering will be encouraged.

Genetic engineering will be applied to pharmaceuticals in particular. The production of the surface antigen of hepatitis B virus and growth hormone in eukaryotic cells are among the practical projects. Human and veterinary products will be developed, with antibiotics and bioreagents receiving priority. A particular effort will be made to produce diagnostic monoclonal antibodies since 70% of the French diagnostic market is currently supplied by German and American concerns. Immunology is also a target for research, with emphasis placed upon gamma interferon and interleukine production. The search will continue for higher quality human and verterinary vaccines through the development of purer and cheaper antigens.

Agriculture is also addressed by the new program. The French goal is to master plant differentiation and regeneration in order to more effectively utilize genetic engineering. A center for plant genetic engineering is being created to aid and coordinate all the projects in this important field.

—Anne Zotov

Pierre Douzou, a key administrator for France's biotechnology program.

INDUSTRIAL MICROBIOLOGY

SCIENTISTS ENGINEER PROTEINS FOR CELLULAR EXPORT

COLD SPRING HARBOR, New York—Several virus proteins that are normally anchored in a membrane have been genetically engineered to proteins that are exported from infected eukaryotic cells. The secreted proteins, which are biologically active, can be purified far more easily and quickly than membrane proteins. The technique should be applicable to many other membrane proteins, facilitating the production of enzymes, receptors, and viral antigens for vaccines.

Eukaryotic cells have evolved a common mechanism for dispatching secretory and some membrane proteins. A short "signal" peptide at the amino terminus, or beginning, of the protein molecule directs the protein to an intracellular membrane and initiates transfer of the polypeptide across the membrane. In the case of secretory proteins, the entire protein is extruded through the membrane and into an extracytoplasmic compartment. In the case of membrane proteins, a hydrophobic "anchor" peptide at the end of the protein serves as a "stop transfer" signal and the protein remains lodged in the membrane.

"In principle, this technique should be able to turn any of these membrane proteins into secreted proteins in any cell, from yeast to human," reports Mary Jane Gething, a staff scientist at Cold Spring Harbor Laboratory in New York. Gething and Joe Sambrook, assistant director of the laboratory, constructed a hybrid vector composed of sequences from SV40 and the gene for the hemagglutinin (HA) protein of influenza virus, then engineered a mutant in which the sequences coding for the hydrophobic anchor of the HA were deleted (Nature, **300:** 598). The mutant protein was synthesized, processed, and secreted into the extracellular medium very efficiently, producing as many as 10^9 HA molecules/cell.

Michael Sveda and his colleagues at the National Institute of Allergy and Infectious Diseases constructed SV40-HA vectors with large (approximately 500 bp) deletions that included the gene sequences coding for the hydrophobic anchor segment (Cell, **30:** 649). These mutant proteins were also secreted, but the secreted form was not structurally identical to the membrane-bound HA, perhaps because some of the deleted polypeptide was necessary for processing of the protein. John Rose (Salk Institute) and John Bergmann (University of California, San Diego) used an approach similar to that of Gething and Sambrook to construct an anchorless mutant of the VSV G protein; this mutant was also secreted from the cells (Cell, **30:** 753).

The idea that removal of the anchor sequence could convert these proteins from a membrane-bound to a secretory form "was pretty much obvious to everyone," states Rose. "I think the problem comes in trying to engineer an extremely high level of expression of the protein. Gething and Sambrook have gotten better results using HA than anyone else." Officials at Cold Spring Harbor Laboratory are investigating patent rights on the techniques that Gething and Sambrook have developed.

Perhaps the simplest application of this technology is the production of viral antigens for subunit vaccines. Gething reports that "we have started to talk to people who are interested in producing flu vaccines about using this method to produce viral antigens." Many viral antigens are proteins similar to flu HA and VSV G protein, and anchorless mutants of such proteins could be an excellent source of antigen for vaccines.

One technical problem with these subunits is that they are monovalent and therefore less immunogenic than the aggregates formed by isolated HA molecules that contain the membrane anchor peptide. "The ease of antigen isolation is secondary to whether the protein is immunogenic," observes Dennis Kleid, a senior scientist for vaccine development at Genentech. Gething does not think that this is a difficult problem: "We can make the monovalent, anchorless HA molecules polyvalent by cross-linking them, or perhaps by other methods. Clearly one needs polyvalent complexes for immunogenicity, but they are not difficult to create. I think that the simplicity of purifying a protein like HA from the medium is much more important than any inconvenience involved in making the proteins polyvalent."

Although the conversion of membrane proteins such as HA and VSV to secretory proteins was conceptually simple, application of this technique to other kinds of proteins may not be. For example, many membrane proteins, such as enzymes and ion channels, have multiple anchor peptides and/or hydrophobic faces that form only when the protein is folded. Engineering secretory forms of such proteins may be difficult.

A more important question, however, is whether cytoplasmic or soluble proteins, such as enzymes, hormones, growth factors, or food proteins, can be converted to secretory proteins simply by adding a signal peptide to the amino terminus of the molecule. In order to answer this question Gething and Sambrook constructed a SV40-HA recombinant vector in which the HA signal peptide coding region was linked to the gene for SV40 T antigen, a non-membrane protein that is normally present in the cytoplasm and nucleus of infected cells. Stressing that the results are very preliminary, Gething reports that the hybrid T antigen is synthesized and transported across an intracellular membrane. They have not yet determined whether the protein is processed and secreted. Both the Cold Spring Harbor group and Ching-Juh Lai's group at the National Institute of Allergy and Infectious Diseases anticipate using the SV40-HA vectors to add the membrane anchor sequences of HA to other proteins, which could then be expressed on the surface of infected cells.

If these experiments are successful, they will form the basis for a major biotechnological advance: a single vector could be used to isolate and identify a gene and its product, direct cells to synthesize vast quantities of the product, then purify it.

Several researchers have suggested a method for exploiting this advance. One would insert the putative gene for a protein, for example a hormone, into the SV40-HA vector containing both the signal and anchor peptide sequences and use the vector to infect eukaryotic cells. If the gene is expressed, the hormone will appear on the surface of the infected cells. Using a fluorescent anti-hormone antibody and a fluorescent-activated cell sorter, one could automatically screen millions of cells and isolate any that are producing the hormone. The next step is to reisolate the vector from the producer cells and remove the sequences that code for the anchor peptide. The modified vector will then direct infected cells to synthesize and secrete the hormone. Experiments to be done in the next six to 12 months should indicate just how realistic this scheme is.

—Mitchel Zoler and Tazewell Wilson