by the assay in suckling mice. This experiment does not violate the research guidelines because it involves transfer of the enterotoxin gene between strains of *E. coli* and not the introduction of a new gene into the species.

Falkow hopes to be able to use the cloned DNA to identify the gene product and learn how it works. He has found, for example, that minicells (see below) bearing the recombinant plasmid contain five or six proteins not observed in minicells having only the carrier plasmid. The next step is to identify which of these proteins are involved in synthesis of the toxin.

Minicells are formed by a mutant strain of *E. coli* that cannot synthesize DNA but that does bud to form small cells without chromosomes. Because these minicells are so much smaller than normal bacterial cells the two can be easily separated by centrifugation. Plasmids are taken into the minicells during budding and function there. Thus investigators can study processes controlled by plasmids in the absence of those under chromosomal direction.

The bacterium *E. coli* is quite simple compared to nucleated cells, and more is known about it than about any other cell type. Consequently, the ability to put eukaryotic genes into *E. coli* should give molecular biologists a way to approach some of the many unanswered questions about the expression of these genes and how the expression is controlled. To do this, investigators must first find out whether prokaryotic enzymes will recognize and respond to the signals that control transcription and translation of the eukaryotic genome. This is also important because, if they do not, a product such as insulin will not be synthesized by bacteria unless the recombinant DNA molecule also includes the appropriate prokaryotic control signals at the correct locations.

Initiation of transcription of at least one kind of eukaryotic DNA—mouse mitochondrial DNA—does occur on the eukaryotic portion of the recombinant molecule in *E. coli* minicells. Cohen and David Clayton, also at Stanford University School of Medicine, formed recombinants between the whole mitochondrial chromosome, which is a circular DNA molecule, and the pSC101 plasmid and cloned them in minicells. They found that portions of the mitochondrial genome were transcribed into RNA's, and the pattern of the RNA's formed indicated that the transcription originated at sites within the mitochondrial DNA itself. Cohen points out, however, that these transcription signals to start do not appear to be the normal ones of the mitochondrial DNA.

Cohen and his colleagues also examined protein synthesis in minicells containing the recombinant molecule and compared it with that of minicells having only pSC101. They found that the recombinant transformed cells contained peptides not produced by the pSC101 transformation and concluded that the peptides were translated from DNA transcripts of the mitochondrial DNA. However, these peptides differed markedly from those normally formed under the direction of the mitochondrial chromosome. Thus, at this time there is no evidence that DNA from higher eukaryotic organisms will be expressed normally in bacteria.

Although most investigators are now using plasmids as cloning vehicles, bacteriophages can also serve in this capacity provided that they are suitably modified. Bacteriophage DNA is larger than most plasmids and will usually be split in more than one place by restriction enzymes. This would obviously complicate the process of constructing a workable recombinant. Mutants with only one or two enzyme-sensitive sites are needed. Moreover, nonessential DNA must be deleted to make room for additional DNA; otherwise, the recombinant would not fit in the protein coat of the phage. Finally, these alterations must not destroy the capacity of the phage to infect and reproduce in bacteria.

Three investigators, Davis, Kenneth Murray of the University of Edinburgh, and Alain Rambach of the Pasteur Institute, have altered bacteriophage lambda DNA so that it meets these criteria. This DNA normally has five sites that are cleaved by Eco RI. The investigators have been able to produce mutants with only one or two. Davis, for example, constructed a mutant with two cleavage sites. Digestion with Eco RI therefore produces three fragments. The small central fragment includes no genes needed for phage propagation. It can be separated from the two larger end fragments. These have the genes required for infection and reproduction in the host bacterium, but when joined together, they form a molecule that is too small to be active. However, if another piece of DNA is inserted between them, a viable phage results. The fact that the phage cannot reproduce unless the DNA is of the correct length provides a mechanism for selection of recombinant molecules. Davis has used this modified phage to isolate ribosomal RNA genes of yeast.

One of the advantages of using bacteriophage lambda DNA is that this genome has been extensively mapped and its control regions identified. It may be possible to construct a recombinant molecule between eukaryotic DNA and a modified lambda DNA in such a way that the expression of the eukaryotic material is controlled by phage genes.

Recently, Philip Leder and his colleagues at the National Institute of Child Health and Human Development further modified the phage developed by Davis in order to make it even more suitable for use as a cloning vehicle. They introduced one mutation that allows the growth of large quantities of the virus in a relatively small volume. Two additional mutations improve the safety of the vector by reducing the possibility of its encountering a suitable host in nature.

Many problems, including the current inability to demonstrate that eukaryotic genes are expressed in bacterial cells, will have to be solved before recombinant DNA techniques find practical application. Most investigators, however, are optimistic that the difficulties can be overcome. Meanwhile, molecular biologists have a powerful tool with which to explore the riddles of gene structure and function.

—JEAN L. MARX

Laser Enrichment: Time Clarifies the Difficulty

For several years, laser techniques have stood out as the most exotic and promising new methods of uranium enrichment. The idea is that monochromatic laser beams can be used to separate isotopes on the basis of miniscule chemical differences long thought to be too small to exploit. The promise is that laser methods could potentially save as much as half the cost and 90 percent of the energy used in present enrichment methods. Many observers have feared that laser methods would be so easy to implement that they might also, as one weapons scientist said, enable people to "build bombs in their basements."

The status of laser enrichment technology has been obscured by the secrecy imposed on the research, in the government weapons laboratories at Los Alamos and Livermore and at the Avco-Exxon Nuclear laboratories where a large amount of industrial research is done. Perhaps the clearest perspective to date was presented last month at an American Physical Society meeting in New York, where representatives from all three laboratories

spoke. While there was no indication that the big laboratory programs are slowing down, the discussions in New York dispelled the idea that laser enrichment is a simple technique that any trained scientist can duplicate with a few store-bought lasers.

The major motivation for the laser development programs is to produce fuel for nuclear power stations. All the present power reactors, except the Canadian CANDU reactor, require fuel that is enriched in ^{235}U, so that shortages of enrichment capacity could slow down and possibly cripple nuclear power development. But the gaseous diffusion method now used for uranium enrichment is becoming increasingly expensive, particularly because it requires prodigious quantities of electricity. Laser and centrifuge methods offer alternative ways to enrich uranium, with much less energy consumption. Still another method, the nozzle process which is reportedly the basis for the enrichment technology West Germany sold to Brazil (*Science*, 30 May 1975), is more energy-intensive than gaseous diffusion.

One comparison of the critical data for laser, centrifuge, and gaseous diffusion plants shows that the laser methods win over the centrifuge by a slight margin in all categories (Table 1). The figures were compiled by Richard Levy of the Exxon Nuclear Corporation and presented in New York. The Avco-Exxon Nuclear partnership just last week announced its intention to build a $15 million test facility for laser enrichment at Richland, Washington. Due to be completed in 1978 or 1979, the facility could be later upgraded into a pilot plant. Application is pending before the Nuclear Regulatory Commission.

The figures for laser enrichment only refer to the type of system that is based on atomic uranium vapor. Atomic enrichment methods are being studied at Livermore, as well as at Exxon-Avco, and molecular methods are being studied at Los Alamos. Estimates of plant costs from Livermore are lower but fairly consistent with the ones in Table 1—as they should be if they refer to basically similar atomic systems. Los Alamos spokesmen have apparently always said that their method had the most potential for radical reductions in size and cost, and their plant projections reflect such optimism. According to Paul Robinson, a plant based on the Los Alamos molecular scheme would cost only 10 percent as much as a diffusion plant, and the size (for a capacity of 6000 to 7000 metric tons) would be only 1 acre.

Just how far have laser technologies progressed? Gaseous diffusion plants have been operated for 30 years, and the total capacity of U.S. plants, now being upgraded, is 17,000 metric tons. Centrifuge technology is being tested in a 25 metric ton pilot plant in Europe, but has not quite reached that stage in the United States. Laser methods, so far as anyone has reported, have produced only 2.5 milligrams of enriched uranium, which now sits in a paperweight on the desk of Robert C. Seamans, Jr., head of the Energy Research and Development Administration.

Table 1. Costs and performance factors for three types of uranium enrichment. The basic measure of uranium enrichment is the separative work unit (SWU); 1000 SWU is equivalent to 1 metric ton. The laser estimates refer to methods for separating uranium atoms, but not uranium molecules. [Source: Richard H. Levy, Exxon Nuclear Corporation]

	laser	Centrifuge	Diffusion
Separation factor	10		1.0043
Energy requirement (kilowatt-hour/SWU)	170	210	2100
Capitol cost ($/SWU)	195	233	388
Economic size (metric tons)	3000	3000	9000
Process area (acres)	8	20	60
Possible completion date	1986	1982	1985

Any technology that produces slightly enriched uranium can also be used to produce highly enriched uranium for weapons, but the practical difficulties vary. Gaseous diffusion has such a small separation factor (the amount by which enrichment is increased) that 1200 stages are needed for reactor fuel and 4000 stages are needed for weapons-grade material. Centrifuges and lasers have much better separation factors. The European centrifuges have an enrichment factor of 1.5 to 2.0, according to Jack Ruina of the Massachusetts Institute of Technology, and the American ones do considerably better, although the sizes and separation factors of the larger American machines are classified secrets. Both laser and centrifuge methods appear to be capable of high enrichment with relatively few stages, and for a small output they would be considerably cheaper than gas diffusion, according to Ruina. But the danger of nuclear weapons proliferation from the spread of laser methods was considered to be no greater than the danger from 15-year-old centrifuge technology, according to the experts who discussed it in New York.

Instead of dwelling on laser enrichment techniques the eminent panel, convened to talk about "Uranium enrichment and arms control," repeatedly gravitated toward the subject of plutonium as a weapons material. Five of the six nations that have tested nuclear weapons built their first bomb from plutonium, rather than enriched uranium, as Herbert York of the University of California, San Diego, noted. India recently showed that it is rather simple to produce plutonium from a CANDU reactor and develop the chemical reprocessing technology to extract it from the irradiated fuel. The consensus of the panel, which also included Ruina and Harold Agnew, director at Los Alamos, was that the principal dilemma for arms control is to find a way to promote nuclear power while inhibiting nuclear arms. Laser enrichment technologies are not only difficult but also expensive and, as one observer noted, "Even a terrorist has to be cost-effective."

Some of the difficulties of laser enrichment were discussed by Richard Levy. In both atomic and molecular schemes, powerful lasers that can be tuned to the proper wavelengths are needed before tons of uranium can be enriched. "This is not a game that you can play with flashlights," Levy said. For the atomic methods, cells hundreds of meters long are needed to absorb all the laser light. Two or more lasers are needed to ionize the atoms. But two-step methods tend to produce a high population of atoms in an intermediate excited state—the condition for lasing. So if a laser enrichment plant is not properly designed, according to Levy, "You may build a factory and get the world's largest uranium laser!" Other problems are to find ways to produce the tons of uranium feed needed for a plant, holding the power costs for vaporization to a minimum, and to devise good engineering methods to condense both the enriched and the depleted uranium at the end of the separation process.

The problems for the molecular approach are considerably different, and in fact the research on the major problem was just declassified in time for the New York meeting, where the secret of the Los Alamos work was disclosed. Not unexpectedly, the news is that the Los Alamos team has been concentrating on uranium hexafluoride (UF_6), which is used in centrifuge and gaseous diffusion enrichment because it becomes a vapor at the low temperature of 57°C.

The problem with UF_6 is that the molecular states are very dense and tend to overlap, so that it is impossible to find any wavelength at which $^{235}UF_6$ absorbs light but $^{238}UF_6$ does not. At familiar temperatures most UF_6 molecules will not be found in the ground state, but will be distributed in a band of some 12,000 excited states, while to get a selective effect by exciting certain states and not others, you need to start with a large number of molecules in a common state. The Los Alamos scheme is to force the UF_6 through an expansion nozzle at supersonic speeds. The gas is drastically cooled on leaving the

(Continued on page 1193)

RESEARCH NEWS

(Continued from page 1163)

nozzle and a "good fraction" of the molecules are left in the ground state.

The next step is to dissociate the $^{235}UF_6$ molecules, using one of several laser arrangements. Unfortunately, the Los Alamos researchers have not had powerful enough lasers at the proper infrared wavelengths to do any actual separation yet. What they presented in New York was an absorption spectrum, for light at a wavelength of about 16 micrometers, that showed a $^{235}UF_6$ feature clearly distinct from several $^{238}UF_6$ features. The spectrum was measured with a tunable diode laser producing only about 100 microwatts of power. The Los Alamos team is now soliciting bids on bigger lasers. They estimate that for early experiments they need at least a few milliwatts at the exact wavelength (still classified), and 100 watts for commercial applications. The molecular methods generally require a second laser of greater power, either ultraviolet or infrared, to dissociate UF_6.

The Los Alamos announcement was less dramatic than might have been expected. Richard Levy characterized it as "an interesting footnote to a process for which there are no obvious barriers to success."

Last year the Los Alamos researchers achieved successful laser isotope separation of sulfur hexafluoride (SF_6). In that case, the proof did not have to be held up for new laser development, because the SF_6 molecule could be dissociated by the infrared light of a carbon dioxide laser—the most powerful infrared laser now made. For uranium hexafluoride, proof of the method awaits the development of a powerful laser at a wavelength further into the infrared than any of the intense lasers now available.

Since atomic methods of uranium isotope separation can be done with lasers off-the-shelf, even though atomic method proponents would like more power, molecular methods seem to be a greater gamble. The appeal is that they eliminate a number of difficulties, such as the corrosiveness of high-temperature uranium vapor, and the tendency of ionized ^{235}U atoms to recombine before they can be isolated. The preparation and separation techniques of the molecular method would be chemical rather than physical, and this feature appeals to those who believe that chemical techniques are better suited for factories where a large flow of materials is essential.

The Los Alamos team is investing much money and manpower in proving that their methods are superior. The next few years should show whether the promise of the molecular approach is realizable.

—WILLIAM D. METZ