Institute estimate that sometime between 1985 and 1992 helium demand will exceed the yearly supply. At that time, the United States will either have to find new supplies or start drawing on its reserves. The Bureau of Mines suggests that natural gas fields with a low helium content are the most likely new supplies, but many competent observers think that the reserves of natural gas now known—whether rich in helium or not—will be exhausted at about the same time. The Bureau of Mines predicts that new sources of natural gas containing helium will be found. In any case, it is possible that by the end of the century the United States will have to rely heavily—if not exclusively—on extracting helium from the atmosphere.

Recovery of helium from the air could have a staggering impact on the environment. Having canceled the helium conservation program, the government has, in effect, abandoned 20 billion cubic feet of helium. To recover that much helium from the air by liquefaction would require 2 years of the country's present total production of electricity. Thermal pollution would be produced by both the generating plants and the helium recovery plants. The Union Carbide Corporation estimates that the thermal pollution from producing 3.8 billion cubic feet of helium per year in the year 2000 would raise the temperature of the entire Mississippi River about 10°F, if it were used for once-through cooling.

Not only may future helium shortages force the country to resort to production methods that are extremely wasteful of energy, but higher costs of producing helium could make fusion power production and superconducting transmission cables less appealing economically, and thus their application might be delayed. Whatever the specific consequences, the government's policy will require future generations to expend a lot more energy to obtain a vital resource—helium—than ought to be necessary.

The final irony of the demise of the government's efforts to conserve helium is that the contracts binding the Department of the Interior are more stringently written than most government contracts. Upon termination, the contracts require payment to the companies for the undepreciated capital costs of the private helium plants. So, whether 20 billion cubic feet of helium is wasted or not, the Department of the Interior will probably end up paying at least half the cost of saving it.

—WILLIAM D. METZ

RESEARCH NEWS

Molecular Probes: A New Way to Study Gene Expression

Although the cells of a multicellular organism have identical genes (DNA sequences that code for proteins) no cell expresses all of its genes. The differences between skin and nerve cells, for example, are believed to result from the expression of different genes by the different cells. No detailed explanation of gene expression exists, but it is now possible to investigate this phenomenon by means of a molecular probe for specific messenger RNA (mRNA) copies of genes. This molecular probe has recently been used to study the expression of hemoglobin genes in developing red blood cells and to study the expression of genes that code for cellular responses to steroid hormones. Many researchers are optimistic that the probe will enable them to determine which cellular components are necessary for gene expression.

The molecular probe for specific mRNA's was developed independently in the laboratories of David Baltimore at the Massachusetts Institute of Technology in Cambridge, Sol Spiegelman at Columbia University in New York, and Philip Leder at the Molecular Genetics Laboratory of the National Institutes of Health in Bethesda, Maryland. It consists of a copy of DNA that is complementary to a specific mRNA and is radioactively labeled. The complementary DNA (cDNA) is transcribed from the mRNA with a reverse transcriptase, an enzyme that transcribes DNA from RNA. When cDNA is added to extracts of cells, it binds to its complementary mRNA. The cDNA that has bound to its complementary mRNA can then be separated by enzymatic degradation of the unbound cDNA. This assay is particularly simple, since the degraded cDNA is soluble in acid, whereas the cDNA-mRNA complex is insoluble in acid.

The development of a cDNA probe for a specific mRNA depends on the isolation of the specific mRNA from which the cDNA is transcribed. Specific mRNA's can as yet only be isolated from cells that produce a large quantity of a specific protein. In some cases, cells can be chemically stimulated to produce large amounts of a protein. For example, hen oviduct cells produce ovalbumin in response to estrogen. The ovalbumin composes 65 percent of all protein synthesized in these stimulated cells. If progesterone is administered after the estrogen, the cells respond to progesterone by producing large quantities of avidin. Bert O'Malley and his colleagues at Baylor College of Medicine, Houston, exploited this regulatory system to show that hen oviduct cells produce these responses to steroid hormones by controlling the transcription of mRNA.

Investigators have speculated that the synthesis of certain proteins could be regulated by substances that control either one of two processes: the transcription of DNA into mRNA or the translation of mRNA into protein. In eukaryotes (nucleated cells), these two processes take place at different times and in different places. It is possible to determine whether protein synthesis is regulated by transcriptional controls, since, if so, different cells should produce different mRNA's.

O'Malley and his associates could study transcriptional controls in hen oviduct cells because they could isolate ovalbumin and avidin mRNA's (by sucrose gradient centrifugation) and could thus transcribe cDNA probes for these mRNA's. The cDNA probes can be used to monitor the appearance of ovalbumin and avidin mRNA's in hormone-stimulated cells and the appearance of these mRNA's can be correlated with the appearance of ovalbumin and avidin. The cells, O'Malley found, re-

sponded to estrogen and progesterone by accumlating ovalbumin and avidin mRNA's and this occurred concurrently with an intracellular accumulation of ovalbumin and avidin. Moreover, he could prevent the responses to the hormone by exposing the cells to chemicals that prevent DNA transcription.

Another type of cell that can be chemically stimulated to produce large quantities of a specific protein is the Friend cell. These are red blood cell precursors (proerythroblasts) that have been transformed by the Friend murine leukemia virus. Friend cells normally contain neither hemoglobin mRNA nor hemoglobin, but when they are exposed to dimethylsulfoxide (DMSO) they produce hemoglobin.

Leder and his colleagues J. Ross and I. Ikawa have been studying hemoglobin synthesis using a cDNA probe for hemoglobin mRNA in Friend cells. They are able to detect ten or more hemoglobin mRNA molecules in a cell but have found no hemoglobin mRNA in Friend cells that had not been exposed to DMSO. After the cells were treated with DMSO, they found between 6000 and 8000 hemoglobin mRNA molecules in each cell and, concurrently, found hemoglobin in the cells.

Proteins Restrict Transcription

It is difficult to propose molecular mechanisms of transcriptional control since such mechanisms would have to explain relations between many seemingly complicated events. For example, the genes for the two amino acid chains that combine to form hemoglobin are located on separate chromosomes. An explanation of hemoglobin transcriptional control would have to account for the simultaneous initiation of transcription from two chromosomes, and thus is far more complicated than explaining bacterial transcription from the single bacterial chromosome. Rather than attempt such an explanation, many investigators have restricted themselves to identifying those cellular components that appear to be necessary for transcription. Thus recent evidence that relates the proteins associated with eukaryotic DNA to transcriptional control has aroused considerable interest.

Indirect evidence that DNA-associated proteins are involved in transcriptional control was obtained by O'Malley in his study of hen oviduct cells. Steroid hormones that enter these cells bind to receptor proteins. The receptor proteins are initially present only in the cytoplasm of target cells for the hormones. The steroid-receptor complex diffuses from the cytoplasm to the cell nucleus where it binds to the chromosomes. After the hormone-receptor complex binds to the chromosomes, hormone-induced changes in transcription are evident, although they have not yet been proved to result directly from the reaction between the receptor and the chromosome. O'Malley believes that certain DNA-associated proteins, the nonhistone or acidic proteins, are involved in the binding of the steroid-receptor complex to the chromosomes because, if the proteins are removed from the DNA, binding is reduced.

Direct evidence that DNA-associated proteins restrict transcription was obtained by Gary Felsenfeld, Richard Axel, and Howard Cedar at the Laboratory of Molecular Biology of the National Institute for Arthritis and Metabolic Diseases in Bethesda, Maryland. This group showed that globin mRNA can be transcribed in vitro from duck chromatin (DNA together with its associated proteins) isolated from immature red blood cells where globin is synthesized. Globin mRNA was not transcribed from chromatin from duck mature red blood cells, where little globin is synthesized, or from duck liver cells, where no globin is synthesized. Since the DNA is the same in the three cell types, the DNA-associated proteins apparently prevent globin mRNA transcription in cells that do not synthesize globin.

Felsenfeld, Axel, and Cedar transcribed the chromatin with bacterial enzymes. Bacterial enzymes differ from those of the duck, but it is not technically feasible to use duck enzymes for transcription in vitro. Nonetheless, the results of the studies in vitro are consistent with observations in vivo. In addition to the fact that transcription in vitro of globin mRNA is apparently restricted to those cells that synthesize globin in vivo, there is evidence that the relative amount of globin mRNA transcribed in vitro approximates the relative amount transcribed in vivo.

Klaus Scherrer and his colleagues at the Swiss Institute for Experimental Cancer Research in Lausanne found that of all the large precursor RNA transcribed in vivo from duck immature red blood cells 1 part in 10^4 is globin mRNA. Felsenfeld, Axel, and Cedar similarly find that globin mRNA composes 1 part in 10^4 of all RNA transcribed in vitro from these cells. This coincidence does not imply that transcription in vitro mimics the situation in vivo, but it rules out gross differences between transcription in vitro and in vivo.

The fact that globin mRNA is only 1 part in 10^4 of all RNA transcribed in vitro makes it difficult to detect this mRNA. It is necessary to use a cDNA probe for globin mRNA in order to obtain definitive results. This technique can be used to detect 10^{-9} gram of globin mRNA.

Since chromatin from duck immature red blood cells acts as a template for globin mRNA synthesis, Felsenfeld, Axel, and Cedar sought to determine whether they could remove the proteins that are associated with this DNA and still transcribe globin mRNA in vitro. They found that they could not detect globin mRNA in the RNA transcribed from naked DNA when they used the same amount of cDNA that they used to detect globin mRNA in the RNA transcribed from chromatin. Felsenfeld believes that this may be due to the fact that many more varieties of RNA are transcribed from naked DNA than from chromatin. The globin mRNA could be present in the RNA transcribed from naked DNA and yet be so diluted as to avoid detection.

Several experiments are possible now that chromatin can be transcribed in vitro and specific mRNA can be detected. For example, it is now feasible to study the mechanism of assembly of chromatin. Specific protein fractions could be added to or removed from DNA, and it may be possible to determine which proteins are necessary for transcriptional controls of gene expression and to study the operation of such controls. John Paul and Stuart Gilmore of the Beatson Institute for Cancer Research in Glasgow reported successful experiments of this kind at the recent Cold Spring Harbor Symposium.

The experiments of Felsenfeld, Axel, and Cedar, of O'Malley, and of Leder demonstrate that transcriptional control is important in cellular differentiation and regulatory processes. Such model systems as the hen oviduct and Friend cells have been described and now minute quantities of specific mRNA's can be detected. Such research promises a better understanding of transcriptional control mechanisms in the near future.

—GINA BARI KOLATA

Additional Readings

1. J. R. Ross, Y. Ikawa, P. Leder, *Proc. Natl. Acad. Sci. U.S.A.* **69**, 3620 (1972).
2. B. W. O'Malley and A. R. Means, *Science*, in press.
3. R. Axel, H. Cedar, G. Felsenfeld, *Proc. Natl. Acad. Sci. U.S.A.* **70**, 2029 (1973).