New Ways to "Mutate" Genes

Preventing gene expression by blocking translation of messenger RNA's may provide a new way of studying gene action in the cells of higher organisms

Molecular biologists who wish to study gene activity in the cells of such higher organisms as frogs, mice, and men have frequently been hampered by their inability to produce gene mutations. They have not been able to use the standard genetic manipulations that have worked so well for dissecting gene function in bacteria. Recently, however, investigators have begun to develop methods that may remedy this deficiency. It may soon be possible to turn off the expression of specific genes in the cells of higher organisms—in effect producing gene mutations at will.

Two related, but somewhat different procedures, which were described at a recent meeting,* aim to block gene expression by inhibiting translation, the second major step in gene expression. The first step is the copying of the gene into messenger RNA (mRNA), after which the nucleic acid message is translated and the protein is made. Translation can be prevented, the new results show, by introducing into cells an RNA complementary in structure to the mRNA for the protein in question. The complementary RNA—or antisense RNA as the material is called—binds to the messenger and prevents it from being translated.

The method under development in Douglas Melton's laboratory at Harvard University is aimed at blocking translation in early embryos. In his initial experiments Melton has used frog eggs, which do not normally make the protein β-globin but will do so if they are injected with β-globin mRNA. Synthesis of the protein can be blocked, the investigator finds, if the eggs are injected first with an RNA complementary to the globin messenger. "The antisense RNA hybridizes to the globin mRNA in the egg and completely blocks its translation," Melton says.

Translation of the β-globin message was not blocked by antisense RNA for a histone messenger, a result indicating that the effect is specific. Melton has not yet shown that the technique can be used to block translation of endogenous egg mRNA's, however.

*The Cetus-UCLA symposium on the Molecular Biology of Development, which was held in Steamboat Springs, Colorado, on 31 March to 7 April.

The Harvard investigator's method depends on the ability to make sufficient quantities of the antisense RNA accurately in vitro. He does this by attaching the corresponding cloned gene, in the inverted configuration, to a highly active and accurate viral promoter, the SP6 promoter from a *Salmonella* phage. Because the gene is inverted, the normally untranscribed strand is copied into RNA, thus producing the complement of the usual messenger for the gene.

In the procedure being developed by Harold Weintraub, Jonathan Izant, and their colleagues at the Fred Hutchinson Cancer Center in Seattle an inverted gene attached to a promoter is also used as the source of an antisense RNA. But these investigators introduce the inverted gene directly into the target cells, which then make the RNA themselves. The Weintraub group has shown that their method works in cultured cells to reduce expression of a transferred gene coding for the viral enzyme thymidine kinase. The effect of the antisense RNA again appeared to be very specific.

"The antisense RNA hybridizes to the globin mRNA in the egg and completely blocks its translation."

So far the Seattle workers do not fully understand the mechanism of the inhibition, Weintraub says. The antisense RNA may act in the nucleus by binding to the newly formed message, either leading to its degradation or preventing its export to the cytoplasm where protein synthesis occurs. Alternatively, the antisense RNA may act in the cytoplasm, directly blocking translation.

The methods developed by the Harvard and Seattle workers have different strengths. Although injected mRNA's may be rapidly broken down, production of the antisense RNA can be maintained more or less indefinitely in cells that have acquired an inverted gene. "The template is always there," Weintraub says.

But, as Melton points outs, the situation in the early embryo, which makes little or no mRNA, requires a different approach. Preexisting mRNA's of maternal origin, which are already present in the egg cytoplasm before fertilization, direct almost all protein synthesis through the cell divisions of cleavage. Nevertheless, commitment of cells to specific developmental fates begins during this early period. "All the important action occurs before transcription begins in the embryo," Melton notes. If translation of the endogenous mRNA's in the embryo can be blocked by injection of the corresponding antisense RNA's, as was that of the injected β-globin messenger, embryologists will have a new tool for dissecting the functions of the maternal mRNA's.

The antisense RNA may not have to block an entire messenger in order to prevent translation. Weintraub and Izant find that viral thymidine kinase synthesis can be inhibited by a DNA construct that specifies an antisense RNA that is only 50 base pairs long and binds to an untranslated RNA segment near the beginning of the mRNA. There are precedents for this type of inhibition in nature. For example, Robert Simons and Nancy Kleckner of Harvard University and Masayori Inouye and his colleagues at the State University of New York at Stony Brook have found that short RNA segments can regulate bacterial gene expression by blocking translation. Recently, the Inouye group has devised a way of artificially inducing the synthesis of the regulatory antisense RNA in bacterial cells, thereby blocking the expression of specific genes.

Weintraub speculates that the short antisense mRNA's might one day be useful in gene replacement therapy, if they can be used to specifically shut off defective genes. Then, a good new gene, which produces a messenger without the binding region for the antisense mRNA and should thus not be affected, could be introduced into the cells to provide the desired protein. However, that type of gene replacement will not be realized in the near future, if ever. Meanwhile, analysis of gene function remains the greatest potential for the antisense RNA methods.—**JEAN L. MARX**