normal children for 5 years before actually planning such an investigation. She and her associates ensured that the children's parents gave informed consent by choosing children whose parents are trained in the biomedical or health professions. They involved the children in the study by asking their opinions of various procedures. For example, the children did not want their blood drawn, so the NIMH investigators measured concentrations of neurochemicals in urine samples instead of in blood. Rapoport and Buchsbaum say the children enjoyed participating in the study and learned a great deal about how biomedical research is done.

Although the NIMH study demonstrates that researchers can no longer use the paradoxical response for clues to the causes of hyperactivity, another study indicates that there may well be specific biological differences between hyperactive and normal children. Mary Waldrop of NIMH and her associates find that hyperactive boys tend to be physically different from normal boys in ways that may mean that hyperactivity results from a congenital defect.

Waldrop and her associates find that hyperactive boys have significantly more minor physical anomalies of certain sorts than normal boys. Consistent with this finding, Patricia Quinn of Georgetown University and Rapoport note that children in hyperactivity clinics tend to have an unusual number of these anomalies and that fathers of these children who report being hyperactive when they were young also have an unusual number of the anomalies. The theory is that these anomalies occur during the first weeks of pregnancy and that whatever causes them could also lead to abnormalities in the development of the central nervous system. For example, children with Down's syndrome have 17 of the anomalies. The anomalies include malformed ears, assymetrical ears, a curved fifth finger, and a wide gap between the second and third toes.

One danger in predicting hyperactivity from the occurrence of minor physical anomalies is that it could become a self-fulfilling prophecy. That is, baby boys with a large number of anomalies would be expected to be hyperactive and would be treated as though they were. This could possibly "cause" the children to be hyperactive. Waldrop points out, however, that she and her associates find very few false negatives.

Girls are far less likely than boys to be hyperactive but are probably equally likely to have minor physical anomalies. Waldrop and her colleagues find that girls with more than the average number of these anomalies are often the very opposite of hyperactive. That is, they are shy, talk very little, and seem overly in control of their movements.

Investigators are still far from pinpointing the distinction between normal and hyperactive children. If the causes of hyperactivity are indeed congenital defects, it is not clear how the disorder may be prevented. For now, researchers are left with drugs and behavioral and educational counseling as means of treating the symptoms of hyperactivity. But progress is being made as researchers continue to question the efficacy of treatments and increasingly come to realize that, in Eisenberg's words, what is usual and customary in medical practice is not necessarily what is safe and useful.

—GINA BARI KOLATA

Gene Structure: More Surprising Developments

Most of what is known about gene expression comes from studies of simple, nonnucleated cells such as bacteria. In these cells, the process is relatively straightforward. First, the DNA of a gene is copied into a corresponding RNA molecule called a messenger (mRNA); then the mRNA directs the synthesis of the appropriate protein, which finally goes about its business as an enzyme or structural component of the bacterial cell. The nucleated cells of higher organisms, however, are much more complicated than bacteria and, consequently, have provided more frustration than information to researchers trying to study how they express their genetic information.

Now that situation is changing. Investigators are beginning to see some progress in their efforts to unravel the secrets of gene expression in nucleated cells. And what they are finding is significantly different from what has been learned about the process in bacteria. A current illustration is the discovery by several investigators that a number of genes from nucleated cells carry within themselves nucleotide sequences (called intervening or spacer sequences) that are not found in the messengers corresponding to the genes. In contrast, bacterial messengers, as far as is known, are direct copies of the genes, without any missing segments. Thus, the nucleated cells apparently have a mechanism, not found in bacteria, for producing mRNA's from which some gene sequences are omitted or deleted.

Similar results were reported last summer concerning the structure of the mRNA's of animal viruses (*Science*, 26 August 1977, p. 853). Because the viruses use the enzymes of the nucleated cells they infect to produce viral components, including mRNA's, investigators hypothesized that both the viral and cellular messengers are synthesized in the same way. At that time, there was already some direct—but still preliminary—evidence supporting this inference, although the researchers studying the structures of the cellular genes and messengers were not yet ready to interpret their findings in that way. Since then, however, an accumulating body of additional evidence has supported the hypothesis.

The work on the structure of eukaryotic (eukaryotes are organisms whose cells are nucleated) genes is proceeding so rapidly that a list of genes found to contain intervening sequences may be out of date before it rolls off the presses. Thus far, spacer sequences have been identified in genes for one of the protein chains (designated the β-globin chain) that form the hemoglobin molecule, in immunoglobulin and ovalbumin genes, and in genes for transfer RNA's (tRNA's) and ribosomal RNA's (rRNA's).

For example, Philip Leder and his colleagues at the National Institute for Child Health and Human Development (NICHD) discovered that two different mouse genes for β-globin contain intervening sequences encompassing some 550 nucleotides. By determining the nucleotide sequence of a portion of one of the genes, they ascertained that the intervening sequence begins immediately after the codon (a sequence of three nucleotides that specifies a particular amino acid) for amino acid 104 of β-globin. Leder and his colleagues also have evidence for the presence of a smaller spacer region near the end of the gene where initiation of mRNA synthesis occurs. Meanwhile, A. Jeffreys of the University of Leicester in England and R. Flavell of the University of Amsterdam in Holland identified a spacer sequence about 600

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nucleotides in length near the codon for amino acid 110 of rabbit β-globin.

Gene sequencing has also been used to confirm the presence of an intervening sequence in an immunoglobulin (antibody) gene derived from a line of embryonic mouse cells. Susumu Tonegawa and his colleagues at the Basel Institute for Immunology in Switzerland, with Walter Gilbert, Allan Maxam, and their colleagues at Harvard University, found near one end of the gene a stretch of 93 nucleotides which is not represented by a corresponding amino acid sequence in the immunoglobulin molecule. The sequence is apparently absent from the mRNA for the immunoglobulin; if it were present, it would be translated into protein structure.

In addition, the Basel investigators identified a longer spacer sequence including about 1250 nucleotides between the genetic regions coding for two portions of another immunoglobulin molecule. Tonegawa had earlier obtained evidence that the two genetic regions were adjacent to one another in the cell line from which this immunoglobulin gene is derived; he was somewhat surprised to find on closer examination that the spacer region separated them.

The evidence for the presence of intervening sequences in the ovalbumin gene of the chicken is not yet as well developed as that for the hemoglobin and immunoglobulin genes; however, two groups of investigators, R. Breathnach, J. L. Mandel, and P. Chambon of the Laboratoire de Genetique Moleculaire des Eucaryotes du Centre National de la Recherch Scientifique in Strasbourg, France, and M. T. Doel, M. Houghton, E. A. Cook, and N. H. Carey of G. D. Searle Research Laboratories in England, have obtained data indicative of spacer sequences.

The mouse, rabbit, and chicken are well advanced on the evolutionary scale, but intervening gene sequences are present in less advanced eukaryotic species as well. In one of the earliest reports of the existence of such sequences, David Hogness and his colleagues at Stanford University Medical School showed that some of the genes coding for the larger of the two rRNA's of the fruit fly (*Drosophila*) contain spacer sequences. These genes, however, are repeated several times in the fruitfly genome and not all the repeated units have the spacer sequences. Consequently, the physiological significance of the spacers in these genes is uncertain.

More recently, Benjamin Hall and his colleagues at the University of Washington sequenced four yeast genes, each of which codes for a different tRNA molecule of the amino acid tyrosine. All four of the genes carry the same 14-nucleotide spacer sequence immediately adjacent to the trinucleotides specifying the anticodons of the tRNA's. (The anticodon is the sequence of three nucleotides that recognizes and binds to the corresponding codon on mRNA.) The insertion does not appear in any of the tRNA's themselves.

Thus, it is clear that the genes of a wide variety of eukaryotic species carry intervening sequences and that the presence of these sequences in genes may be a general phenomenon. What they are doing there is less clear. Virtually all of the investigators have speculated on the possible functions of the spacer sequences, but at present there is little evidence either to support or to invalidate the speculations.

One possibility is that the intervening sequences are involved in turning gene expression on or off. Jeffreys and Flavell, however, have evidence that spacer sequences are present in genes from cells that do not synthesize hemoglobin as well as in genes from cells producing the protein. They thus conclude that it is unlikely that the spacers inactivate the gene in the nonsynthesizing cells.

Another possibility is that the sequences help to regulate protein synthesis at some stage after messenger synthesis. Research on the animal virus messengers suggests that the intervening sequences are copied into RNA, and that they are subsequently excised during the formation of active messengers. The viral spacer segments are in genetic regions thought to be involved in the control of messenger and protein synthesis and not in the regions coding for protein structure. A significant difference in the present work is that the spacer regions of the cellular genes are often found within the structural gene segments. They could still serve as signals of some sort, however, and their excision from RNA molecules could provide another step where protein synthesis might be regulated.

There is evidence from Leder's laboratory that the spacer segments of the β-globin gene are transcribed into RNA and then excised. Other investigators had previously shown that the mRNA for this protein is formed from a precursor RNA that just happens to be the right size to contain both the structural and spacer sequences. Leder, with Shirley Tilghman and David Tiemeier of NICHD and Peter Curtis and Charles Weissmann of the Institut für Molekularbiologie in Zurich, Switzerland, now find that the full length of the gene hybridizes with the precursor without any loops of uncomplementary DNA left over; if the precursor lacked the spacer sequence, there would be loops of leftover DNA. Moreover, the bases on the two ends of the precursor appear identical to those on the ends of the messenger itself. This indicates that the ends are not cut off when the messenger is formed.

It remains to be seen whether the intervening sequences of the other genes are also copied into RNA. Alternative mechanisms can be postulated to account for the absence of the spacer region. For example, the enzymes that synthesize the messengers might simply skip over the spacer segments of the gene. Or the messengers might be synthesized in separate segments that are joined later.

Nevertheless, the findings of the Leder group support the hypothesis that the mRNA's of nucleated cells are synthesized by way of a series of larger intermediates that are subsequently shortened to form the active messengers. The surprising aspect of the work is that the shortening occurs in the interior of the molecule and not on the ends.

Because the intervening segments of the β-globin and other genes are located within the structural regions, excision of the inserts and the rejoining of the RNA pieces must be performed with great precision. The loss of a single base, for example, could completely scramble the rest of the message. The need for enzymes to carry out the excision and joining and for signals by which these enzymes recognize the region to be cut out and the ends to be tied together are additional factors that must be considered if the mechanism of gene expression in nucleated cells is to be understood. As Leder points out, eukaryotic gene expression appears more complicated than investigators would have predicted on the basis of their knowledge of bacteria.

If that is the case, then there are implications for research with recombinant DNA. One of the goals of the research is the development of bacteria as factories for manufacturing large quantities of such substances as hormones and antibodies that can now be obtained only in small amounts from human sources. Since it is unlikely that bacteria will have the more complex machinery needed for the expression of eukaryotic genes, that goal may be impossible unless the genes can be altered to resemble those of bacteria and thus be subject to the same controls. On the bright side, for those who are still concerned about the potential danger of recombinant DNA research, the chances that expression of a eukaryotic gene will produce a dangerous pathogen of some sort are also less likely.—JEAN L. MARX