Isolating Giant Globin Precursors

from our Molecular Genetics Correspondent

THAT messenger RNA is cleaved from a large precursor molecule in the nucleus of eukaryotic cells has been suspected for some time ; and the discovery that a sequence of polyadenylic acid (poly(A)) is added to the 3′ end of the large heterogeneous nuclear RNA (Hn RNA) and then appears in cytoplasmic messenger RNA suggests a mechanism for this processing. But confirmation of the mechanism inferred from experiments on cellular populations of RNA molecules can come only from experiments which follow the production of an individual messenger from its transcription through to its translation. An approach to such experiments, fittingly performed with the globin synthesizing system of reticulocytes which has laid the basis for many advances in RNA metabolism, has been reported by Imaizumi, Diggelmann and Scherrer in the *Proceedings of the National Academy of Sciences* (**70**, 1122 ; 1973).

Pulse labelling with uridine has revealed that almost all of the RNA synthesized in the nucleoplasm (that is, to exclude the ribosomal RNA synthesized at the nucleolus) of many eukaryotic cells is unstable and is degraded rapidly within the nucleus. This RNA consists of a population of molecules very much larger than messenger RNA ; hybridization experiments have suggested that all the sequences of cytoplasmic RNA are found in the nuclear RNA, but that many of the sequences of nuclear RNA are restricted to the nucleus and are never transported to the cytoplasm. This has suggested models for the production of messenger RNA in which a larger precursor is the immediate product of transcription ; most of the precursor is then degraded rapidly but a small region from the 3′ end, marked by its possession of poly(A), is conserved and transported to the cytoplasm where it functions as messenger and is translated by the ribosomes.

Globin messenger RNA can be isolated from the polysomes of reticulocytes as a fraction of size about 9S. The presence of poly(A) at the 3′ end of the molecule has proved a useful tag for the synthesis of its complement ; no less than three research groups, seeing the potential of this discovery, simultaneously announced last year a sequence of oligo(dT) to the poly(A) and had then used the reverse transcriptase enzyme of RNA tumour viruses to extend this primer into a DNA complement to the globin message. Production of this "anti-messenger" has now become almost a routine technique ; and Scherrer and his colleagues have put it to good use as a probe for globin sequences in nuclear RNA of duck reticulocytes.

When the anti-messenger DNA is annealed with an excess of RNA, the rate of reaction depends on the concentration of globin sequences in the RNA population. The kinetics of reaction of an unknown preparation which is under test must be compared with a standard of known complexity ; Scherrer and his colleagues have assumed that the 9S RNA fraction contains two types of globin RNA sequences, representing the α and β-chains, and on this basis have calculated the number of globin sequences in fractions of Hn RNA isolated from a sucrose gradient. Essentially, this technique calculates the length of the Hn RNA which contains an equivalent of globin mRNA ; by comparing this length with the physical length of the Hn RNA molecule, and assuming that only one globin sequence is present in any one Hn RNA molecule, it is possible to deduce the number of Hn RNA molecules which carry globin sequences. The proportion varies from 10–50 per cent in the different size classes of the Hn RNA. This is, of course, a very high proportion, as might be expected from the concentration of the reticulocyte in synthesizing haemoglobin.

The direct and most important conclusion to be drawn from these experiments is therefore that the molecules of Hn RNA contain the sequences of globin mRNA ; the specificity of the reaction between Hn RNA and anti-messenger was confirmed by the failure of the anti-message to hybridize to fractions of RNA other than the Hn RNA and the globin messenger itself. This suggests that cleavage of the large Hn RNA molecules provides the precursor of the globin sequence. But as Scherrer and his colleagues carefully point out, the demonstration that the information for globin is carried in Hn RNA does not prove its direct utilization as the message ; it remains possible, although perhaps unlikely, that some further stage of information transfer intercedes between Hn RNA and mRNA.

The characterization of globin messenger sequences in these enormous Hn RNA molecules emphasizes the discrepancy in size between mRNA and Hn RNA. The largest Hn RNA molecules which carry globin mRNA are of one hundred times longer than the message ; even the shortest molecules of Hn RNA are some ten times the length of the globin RNA sequence. The relationship between these different size classes of Hn RNA is not clear, but it is possible that the smaller molecules of Hn RNA are already the products of cleavage from the large transcripts. The function and structure of the sequences of the Hn RNA which are lost during maturation remain mysterious ; presumably, they must have some specific role in gene expression.

The isolation by hybridization with globin anti-message of the molecules of Hn RNA which contain the globin RNA sequences offers the prospect that the sequences of the precursor which are degraded can be studied. The demonstration that globin sequences are present in Hn RNA may therefore prove only the first step in the unravelling of the processes by which messenger RNA synthesis is effected and controlled in eukaryotes.

GENE TRANSFER

Using Chromosomes

from our Cell Physiology Correspondent

ALMOST two years ago Merril, Geier and Petricciani (*Nature*, **233**, 398 ; 1971) reported that genetically defective human cells could be repaired with the genome of bacterial cells. This report at the time understandably aroused considerable scepticism. Another team, also from the National Institutes of Health in Bethesda (McBride and Ozer, *Proc. US Nat. Acad. Sci.*, **70**, 1258 ; 1973), now suggests that it is possible to transfer genetic information from one type of mammalian cell to another by means of isolated chromosomes. "Feeding" chromosomes from normal cells to cells with a genetically inherited metabolic deficiency evidently results in the correction of the deficiency. The question of "gene modification" is raised once more ; this new method of McBride and Ozer, however, seems more plausible than that of Merril and his colleagues.

The well known A_9 cells, which lack the enzyme hypoxanthine phosphoribosyl transferase ($HPRT^-$) and which were derived from mouse L-cell fibroblasts, were used in the experiments, the chromosomes coming from either Chinese hamster fibroblasts or HeLa cells. When cells and chromosomes are mixed and then put into a selective medium in which A_9 ($HPRT^-$) cells cannot grow, clones of dividing cells which have by some means or another acquired the capacity to synthesize the hypoxanthine phosphoribosyl transferase enzyme can be isolated. The frequency with which these clones arise is not particularly high, but all the necessary control experiments suggest that the appearance of enzyme is not merely the result of genetic reversion.

In the first place the enzyme from A_9 ($HPRT^+$) clones obtained by treatment with hamster chromosomes was chrom-