It has also been demonstrated that the onset of haemoglobin synthesis is characterized by the preliminary appearance of globin mRNA within the cells. Leder calculates that the Ikawa cells used by his group contain less than six copies of globin mRNA per cell before induction (the limit of detection with their cDNA) and more than 6,000 copies per cell afterwards. The appearance of the globin mRNA is sensitive to actinomycin D and therefore represents the product of new transcription rather than some post-transcriptional processing within the cells. In Friend cells, however, where the uninduced cell population contains a low percentage of haemoglobinized cells, Paul's group find that although the polysomal globin mRNA content is much higher in the induced cells, the nuclear globin mRNA content, albeit very low, is the same before and after induction. This might suggest that here the primary block in globin gene expression is a post-transcriptional processing event. Whatever the outcome, it is clear that studies such as these are going to prove archetypal for other differentiation systems. There will no doubt be endless parallel studies performed for every mRNA purified within the next few years.

Paul's group have made further use of the globin cDNA to assay for transcription of globin mRNA from isolated chromatin. He presented convincing evidence to show that globin mRNA may only be transcribed from chromatin derived from an erythropoietic tissue and that the non-histone proteins of the chromatin are responsible for conferring this tissue specificity upon the DNA. The formidable task of purifying the tissue-specific non-histone proteins lies ahead.

These *in vitro* transcription studies were done using the *Escherichia coli* RNA polymerase, "the best mammalian RNA polymerase to date" (Paul) and certainly it was clear, from the careful, thorough work described by Dr P. Chambon (Institute of Biological Chemistry, Strasbourg) on the mammalian enzymes, that the exact requirements for optimal transcription have not yet been found. None of the three polymerases purified by Chambon can transcribe a regular double stranded DNA, probably all of them only initiating at nicks or denatured regions in the DNA, and yet at the same time chromatin is also a very poor template. Chambon has searched the missing sigma-type initiation factors without success and it thus seems possible that the exact structure of the DNA: protein complex in euchromatin is critical for polymerase initiation but is lost in conventional chromatin preparation. The effects of the non-histone nuclear proteins upon transcription are currently being investigated in this respect.

In a second lecture, Dr H. Lodish discussed the work done in his laboratory on the mechanism of mRNA synthesis in the slime mould *Dictyostelium discoideum*, one of the simplest eukaryotic organisms. Using the binding of the poly(A) tail to poly(U) filters as a basic procedure to quantitate nuclear and cytoplasmic mRNA, it has been shown that in this organism the nuclear precursor to cytoplasmic mRNA is not a high molecular weight RNA, but rather a pre-mRNA molecule about 300 nucleotides longer (20% of the total molecule) than the cytoplasmic form. Hybridization data suggest that these extra nucleotides are repetitious sequences, located at the 5′ end of the molecule. From attempts to transcribe mRNA in isolated slime mould nuclei, it became clear that the nuclear pre-mRNA and cytoplasmic mRNA both contain two distinct regions of poly(A) sequences at the 3′ terminus. One of these, a stretch of about twenty-five A residues, is transcribed from similar poly(T) stretches within the DNA, whereas the second region seems to be a conventional mRNA poly(A) tail of about 100 residues which are added post-transcriptionally. Isolated nuclei were found to make mRNA containing the shorter A sequence, but to be deficient in adding the post-transcriptional poly(A).

The meeting closed with a report by Dr G. Dixon (University of Sussex) on the biosynthesis of protamine, the protein used to package DNA within the sperm nucleus. Purification of the protamine mRNA is well advanced but was initially misled, because the protamine mRNA behaves as a much smaller molecule than one would predict from the protein size—probably as a result of extensive secondary structure in the mRNA. Surprisingly, much more protamine mRNA is present in the supernatant fraction than in the ribosomes, a finding which may indicate some translational control within the system.

HAEMOGLOBIN SYNTHESIS

Message in Medium

from our Molecular Genetics Correspondent

WHEN the enzyme reverse transcriptase of RNA tumour viruses was first discovered, it was hailed for the new insights it would bring to tumour virology. Yet already this enzymatic activity is making invaluable contributions to another topic, that of the metabolism of messenger RNA. Because of the complexity of the mammalian genome, the isolation of individual genes is not usually possible and so measurements of their transcripts by RNA–DNA hybridization cannot be made. In a series of experiments last year, however, three research groups announced that they had used reverse transcriptase to synthesize a DNA complement to globin mRNA. This anti-messenger has already been used as a probe to follow globin mRNA synthesis in reticulocytes and is now used by Housman *et al.* (*Proc. natn. Acad. Sci. U.S.A.*, **70**, 1809 ; 1973) and Kacian *et al.* (*ibid.*, 1886) to pin down the molecular defect in α and β thalassaemias.

Thalassaemic diseases in humans comprise inherited disorders of haemoglobin synthesis in which either the α- or β-globin chains may be reduced in amount or absent. The genes for α and β thalassaemias seem to be those which code for the globin chains, so that the defect might lie in either the production or ability to be translated of messenger RNA. Indeed, earlier experiments have shown that protein synthesizing systems *in vitro* are marked by a reduction in synthesis of the affected globin chain. Because the chains which are synthesized are normal in amino acid sequence, the defect must be quantitative and not qualitative.

The messengers which code for the α- and β-globin chains of rabbit reticulocytes can be isolated in comparatively purified states from reticulocyte polysomes and can then be used as a template to synthesize the complementary DNA anti-messenger. Evolutionary restraints on the sequences of globin proteins have fortunately ensured that there is sufficient similarity between man and rabbit for the rabbit anti-messenger to hybridize specifically with the human messenger ; and there is virtually no cross reaction between α-globin and β-globin sequences.

When messenger RNA was extracted from the reticulocytes of normal humans, the assay with α and β anti-messengers showed a 1 : 1 ratio of the two types of messenger. But in patients with α thalassaemia, the ratio of α : β chains was greatly reduced ; and in patients with β thalassaemia, the reverse relationship was found, with a large reduction in the number of β messengers. Of course, all thalassaemias need not necessarily result from the same molecular defect but these results show that in at least some (and very possibly all) thalassaemias the primary defect lies in an inability to synthesize the messenger for one of the two globin chains. Further changes in haemoglobin synthesis may then result from possible feedback interactions between the production of the two classes of chain. The primary defect might lie either in transcription itself or in processing of the giant precursor molecules from which globin messengers are cleaved ; further probes with the anti-messenger can probably distinguish between these two situations.