Messenger RNA in Animal Cells

R. WILLIAMSON

The Beatson Institute for Cancer Research, Glasgow

It is difficult to believe that only 2 years ago, the very existence of messenger RNAs in animal cells was doubtful, and no definitive example of an isolated and purified mRNA could be given. Since that time, the mRNAs for globin (from rabbit, man, mouse, duck, and guinea pig), lens crystallin (chick and cow), ovalbumin, myosin, and silk fibroin have been isolated and their identities confirmed, and histone, protamine, and tubulin mRNAs have been putatively identified. It can be stated with a degree of certainty that if a laboratory has access to a tissue making large amounts of a single protein, and an assay for that protein which is both unique and sensitive, it should be possible to isolate and characterize the mRNA for that protein.

Messenger RNA was first identified in bacterial systems by its rapid labelling with radioisotopes, a rapid turnover relative to ribosomal RNA and 'DNA-like' base composition (Gros *et al.*, 1961; Jacob and Monod, 1961). Although similar criteria have been applied to mRNA in some mammalian tissues, such as liver, in general mRNA in a differentiating cell need not differ in labelling or base composition from other RNA species. Therefore attempts were made to obtain stimulation of the synthesis of a specific protein in a mixed cell-free system. It is essential that the cell-free protein synthesis system used should not normally be capable of synthesizing the protein, for there are many factors which can stimulate endogenous protein activity apart from mRNA. An unambiguous assay system for the protein is also necessary, to distinguish it from endogenous protein synthesis characteristic of the cell-free system.

Globin Messenger RNA

The search for globin mRNA has been the focus of attention in this field for many years. The erythropoietic system is medically relevant and haemo-

globin is a well characterized and easily obtainable protein of known amino-acid sequence. A homogeneous tissue (reticulocytes) active in haemoglobin biosynthesis and making few other proteins is available from many mammals and even (in small amounts) from humans. Embryonic and adult precursor cells from liver and marrow can be isolated (although both are difficult to work with), and there are RNA tumour viruses (Friend and Mirand viruses) which cause cells to differentiate along a haemopoietic pathway after infection. There is a specific hormone, erythropoietin, which also switches stem cells to erythropoiesis. Therefore it is remarkable that it was only in 1968 that the first tentative identification of globin mRNA was achieved, and only in 1971 that mRNA for both α - and β -chains was demonstrated beyond doubt.

Early experiments attempted to persuade ribosomes from the bacterium *E. coli* to translate mRNA for globin, or measured messenger activity by stimulation of total radioactive amino acids incorporated. Both approaches failed. Bacterial ribosomes will not translate mRNA from animal sources, because the initiation tRNA, although similar, differs sufficiently so that it cannot form a functional complex with the ribosome and mRNA. Amino-acid incorporation in itself is a poor measure of messenger activity. Many RNA molecules stimulate amino-acid incorporation non-specifically, in part by protecting endogenous mRNA from ribonuclease action. Ironically, the one type of RNA which often does not stimulate total amino-acid incorporation is messenger RNA, especially when endogenous protein synthesis is at a high level, for it competes for ribosomes with endogenous mRNA. Translation of added mRNA is less efficient than endogenous protein synthesis, and thus total amino-acid incorporation decreases, although the new specific protein coded for by the added mRNA is made.

Received 10 March 1972.

Because of our inability until recently to obtain the demonstrable synthesis of globin in mixed protein synthesizing systems, it was necessary to rely upon indirect criteria in attempting to identify mRNA. The molecular weight of the globin protein chain is known to be approximately 16,000, and since 3 nucleotides code for each amino acid, it can be calculated that the molecular weight of the mRNA would be 160,000, with perhaps a bit added for initiation and termination sequences. The major RNA species found in the cytoplasm (where protein synthesis occurs) are those associated with ribosome structure (sedimenting at 28S, molecular weight 1.53 million; 18S, molecular weight 0.65 million; and 5S, molecular weight 40,000) and the amino-acid transfer RNAs (4S, molecular weight approximately 25,000). The messenger would be found between the 5S and 18S ribosomal RNAs, and in this region there are several components in reticulocytes, some of them degradation products caused by ribonuclease contamination. The species found most consistently are those at 12S (approximate molecular weight 370,000) and 9S (approximate molecular weight 200,000), with a trace of 7S (55,000).

It was thought likely that the messenger RNA might label at a different rate *in vivo* from the structural RNAs, and it was found that the 9S became labelled with ^{32}P -phosphate after intravenous injection into rabbits to a higher specific activity than 28S, 18S, 12S, or 5S RNAs (Chantrenne, Burny, and Marbaix, 1967; Labrie, 1969). Polyribosomes, the active structures in protein synthesis, are connected by a 'naked thread' of mRNA, which is susceptible to low concentrations of added pancreatic RNase, breaking them to single ribosomes. Such a treatment causes the 9S RNA to disappear, as does sonication, which also breaks up polysomes to monosomes (Chantrenne *et al*, 1967; Williamson, Lanyon, and Paul, 1969). Chantrenne and his colleagues also showed that treatment of reticulocyte polysomes with a chelating agent, such as EDTA or pyrophosphate, removes magnesium and causes the polysomes to break into ribosomal subunits (60S and 40S) and releases 9S RNA complexed with an equal amount of protein. This '14S mRNP' also is labelled more rapidly with ^{32}P and is broken down by very low levels of RNase or sonication. All of this circumstantial evidence points very strongly to the 9S RNA being the mRNA for globin, but the substantive proof, translation in a cell-free system, still eluded research groups in this field.

Advances in technique, in particular larger and faster ultracentrifuge rotors and efficient methods of

inhibiting ribonucleases, and the realization that bacterial protein synthesis may have quite different initiation factor requirements from animal systems, led to the successful direct proof of globin messenger RNA activity. The first such demonstration was by Schapira, Dreyfus, and Maleknia (1968), but the RNA fractions used were impure, the system (a rabbit α -chain globin mutant) somewhat unfamiliar to most investigators, and the radioactivity incorporated quite low. In 1969, Lockard and Lingrel published a more convincing demonstration of the synthesis of mouse globin β -chain in a rabbit lysate system, but it was only in 1971 that Lingrel and his colleagues (Lingrel *et al*, 1971; Lockard and Lingrel, 1971; Mathews, Osborn, and Lingrel, 1971) showed that the 9S RNA isolated from reticulocytes of rabbit, mouse, or guinea pig all contained full mRNA activity for both α and β globin chains. This was followed by demonstration of human globin mRNA activity (Benz and Forget, 1971; Nienhuis and Anderson, 1971) and duck globin mRNA (Pemberton *et al*, 1972). A typical experiment from the author's laboratory, based on the experimental procedures of Lingrel *et al* (1971), shows the results obtained for mouse globin mRNA in a duck reticulocyte lysate system (Fig. 1). As can be seen, the basic requirement is the ability to identify unambiguously the product of the added messenger RNA in the presence of a vast excess of protein synthesized by the cell-free system using endogenous messengers. Although some success has been obtained using purified systems, most of the active translations with added mRNAs have been with lysates, that is, cell homogenates centrifuged at 20,000*g* for 20 minutes or so to remove nuclei, mitochondria, and lysosomes.

It is now possible, using the zonal ultracentrifuge, to isolate milligram quantities of globin mRNA (Williamson *et al*, 1971). This has permitted an accurate estimation of its molecular weight as $220,000 \pm 10,000$ (Blobel, 1971; Gaskill and Kabat, 1971; Williamson *et al*, 1971). On high concentration polyacrylamide gel electrophoresis, the mRNA splits into two major and several minor bands, and from the labelling pattern and globin synthesis directed by them, we have suggested that they represent the mRNAs for α and β globin chains (Williamson and Morrison, 1971).

It will be noted that the isolated globin mRNA exceeds by approximately 30% the length necessary to code for the globin chain (220,000 to 160,000). It may be that the excess length is due to the presence of the A-rich sequence at the 3'-end (Lim and Canellakis, 1970; Burr and Lingrel, 1971; Molloy *et al*, 1972). However, recently human globin

FIG. 1. The synthesis of mouse globin directed by mouse mRNA added to a duck reticulocyte lysate. Conditions are as given in Lingrel *et al* (1971). The optical density trace of the duck and mouse globins eluting from the carboxymethyl-cellulose in a salt gradient is given by the solid line. The incorporation in the absence of added mRNA (into duck globin only) is shown by (---); incorporation after addition of 10 μ g purified mouse globin mRNA from reticulocytes using an identical lysate is shown by (- · - · -). Incorporation into both mouse globin chains occurs after addition of mouse mRNA but not in the control.

mutants have been isolated which exceed the normal polypeptide chain length by either 31 residues (haemoglobin Constant Spring, α -chain [Milner, Clegg, and Weatherall, 1971]) or 10 residues (haemoglobin Tak, β -chain [Flatz *et al*, 1971]). The extra amino acids would not be expected to be coded for by an A-rich sequence, and the size of the mRNA in these mutants is awaited. If it is the same size (9S) as normal globin mRNA, then either the A-rich region does not follow on from the termination triplet or the non-coding sequence can be drastically altered without affecting translation.

One of the most remarkable demonstrations of mRNA activity has been by direct injection into *Xenopus* oocytes (Lane, Marbaix, and Gurdon, 1971). As little as 1 μ g of rabbit globin mRNA can be easily detected by this technique, and globin biosynthesis continues for many hours after micro-injection. Not only does this elegantly prove the messenger character of the RNA (for *Xenopus* oocytes are certainly unable to make rabbit globin in the absence of injected mRNA) but also shows that there is the capacity for protein synthesis when free mRNA is added, that is, availability of messenger is at least one control mechanism in determining which proteins are synthesized.

Thalassaemia is a haemoglobinopathy marked by an imbalance in synthesis of normal globin chains. In the most common form, β -thalassaemia, there is normal α -chain synthesis but an absolute decrease

in the production of β -chains, leading to a characteristic anaemia. The condition is hereditary, and it is suggested that the primary gene defect is either decreased transcription of β -chain mRNA, or the synthesis of an mRNA defective in the initiator region, so that it starts protein synthesis less effectively than normal β -chain mRNA (Bank and Marks, 1971). Ribosomes isolated from thalassaemics translate added rabbit globin mRNA normally, making α - and β -chains in the usual ratio, making it unlikely that the ribosomes are defective (Nienhuis, Laycock, and Anderson, 1971). The human globin mRNA isolated from thalassaemic reticulocytes, on the other hand, reflects the clinical picture, coding for fewer α -chains than β even when added to a normal rabbit cell-free globin synthesizing system (Benz and Forget, 1971; Nienhuis and Anderson, 1971).

It is normally assumed that mRNA is polysome-associated; in fact, this is one of the original criteria proposed by Jacob and Monod (1961). However, in the case of human reticulocytes, over 50% of the mRNA for globin is found in the post-ribosomal supernatant (Benz and Forget, 1971); in the case of mouse and rabbit reticulocytes the proportion is much smaller, but appreciable amounts are still found (R. Williamson and C. Hale, unpublished results). It is not known whether this represents mRNA which is no longer able to attach to ribosomes in the cell, or messenger temporarily detached from ribosomes during recycling.

Other Specific Messenger RNAs

The isolation of several other mRNAs has been reported (Table I). In every case a cell type existed which was available in large quantities synthesizing predominantly the protein in question: this could either be a tissue (reticulocytes, silk glands, lens), a tumour line (immunoglobulin, histones), or a hormone-stimulated tissue (ovalbumin). Normally differential labelling with radioisotopes was used as a secondary method of identifying the mRNA, but primary identification depends upon specific protein synthesis on addition of the mRNA to a cell-free system, usually from rabbit reticulocytes or Krebs ascites cells. There does not appear to be a great deal of ribosomal specificity for mRNAs, since rabbit reticulocyte lysates will translate mRNA for ovalbumin or lens crystallin or immunoglobulin, while immature *Xenopus* oocytes will translate rabbit or mouse globin mRNA. Protein identification has been by immunoelectrophoresis, gel electrophoresis, immune precipitation, column chromatography, and amino-acid sequencing. It is obvious that controls must be included to ensure that assays are negative in the absence of added mRNA.

All experiments to date isolating specific mRNA have relied upon the availability of cells or tissues synthesizing primarily a single well characterized protein or group of related proteins. It is worth considering, because of its clinical implications, approaches to isolation of a single mRNA present in small amounts in the presence of a large excess of other mRNAs. It is possible to precipitate polyosomes synthesizing a specific protein, using antibodies to that protein, in the presence of other polysomes (Clayton, Truman, and Campbell, 1972). This should permit the isolation of the corresponding mRNA directly from the polysomes after gel electrophoresis in the presence of sodium dodecyl

sulphate, which dissociates the protein from RNA. The success of this technique depends upon the presence of antigenic groups in incomplete protein molecules. A second, more hypothetical approach, is typified by the isolation of bacteriophage genes by DNA-RNA hybridization. Assume 2 populations of RNA are available, one containing the mRNA in question and the other lacking it but otherwise similar (say, the mRNA for the enzyme hypoxanthine-guanine phosphoribosyltransferase from controls and from Lesch-Nyhan syndrome patients). If a DNA copy is transcribed from the RNA mixture minus specific mRNA, and this DNA is hybridized to the total RNA mixture containing mRNA, all species bar the specific mRNA will hybridize. There are many obvious permutations of this technique, but they depend upon the availability of complementary transcripts from RNA mixtures.

Nucleotide Sequences and Gene Frequencies

It is now accepted that the 'triplet code' for amino acids is substantially correct and universal, but there is still a great deal of interest in the exact nucleotide sequence of animal mRNAs, both to determine whether control of gene expression occurs at this level (for instance, by the availability of transfer RNAs for some triplets rather than others) and to find the nature of the initiation and termination signals.

In a beautiful piece of inductive reasoning, Suzuki and Brown (1972) calculated from the repetitive amino-acid sequence of silk fibroin (a rather poorly defined protein) that the corresponding mRNA would have a particularly high content of guanine (G) residues, but no tetra- or hexanucleotides in a T-1 ribonuclease digest (which splits RNA bonds only after a G-residue). An RNA molecule of molecular weight approximately 5 million was isolated with just the predicted properties from the

TABLE I

Protein	Source	Size	Reference
Globin	Human	9S	Benz and Forget (1971)
Globin	Rabbit	220,000 (9S)	Nienhuis and Anderson (1971) Chantrenne <i>et al</i> (1967) Schapira <i>et al</i> (1968) Lingrel <i>et al</i> (1971)
Globin	Mouse	220,000 (9S)	Williamson <i>et al</i> (1971)
Globin	Guinea pig	9S	Lingrel <i>et al</i> (1971)
Globin	Duck	210,000	Pemberton <i>et al</i> (1972)
Fibroin	Silkworm	45S-65S	Suzuki and Brown (1972)
Ovalbumin	Chick	approx. 14S	Rhoads, McKnight, and Schimke (1971)
Myosin	Chick	approx. 26S	Heywood (1970)
Lens crystallins	Cow	10S-15S	Mathews <i>et al</i> (1972)
Lens crystallins	Chick	10S-15S	Williamson, Clayton, and Truman (1972)
Immunoglobulin (K-41)	Mouse	9S-13S	Stavnezer and Huang (1971)
Histones	Human (HeLa)	7S-9S	Borun, Scharff, and Robbins (1967)
Microtubule protein ('tubulin')	Sea urchin	Not given	Raff <i>et al</i> (1972)
Protamine	Trout	4S-5S	Gilmour and Dixon (1972)

posterior silk gland at the right developmental stage of the silkworm's life. Although the most common triplets for some amino acids can be determined for this RNA, it is far too complex for a total analysis at this time.

Investigation of mRNAs for smaller, defined proteins has been slow. Globin mRNA is a mixture of messengers for the α - and β -chains as isolated, and while techniques (particularly using the isoleucine antagonist O-methyl-threonine to create a 'block' at the first i-leu triplet where ribosomes 'pile up') exist to separate the two messengers, they are difficult and usually only give very small yields (Hori and Rabinovitz, 1968). The 3' end of globin mRNA is an equimolar mixture of an A-sequence 5 or 6 bases long (Burr and Lingrel, 1971), and it is thought likely that there is an A-rich region somewhere else in the molecule as well. However, since this region only contains 70% A, it may be resistant to RNase because of secondary structure rather than base sequence.

It is known that the mRNAs for globin contain considerable secondary structure (Lingrel *et al*, 1971; Williamson *et al*, 1971). It is intriguing to speculate whether animal mRNAs, like viral RNA sequences, will prove to be extensively base paired even in coding sequences (Adams and Cory, 1970).

If a truly pure messenger RNA for a single protein is available, it should be possible to determine the extent of specific base matching with the homologous DNA and from the saturation value, determine how many genes hybridize to the messenger and thus code for it. Unfortunately, this approach is far more difficult with animal cells than bacteria, for the animal genome is very complex and the DNA sequences not only hybridize to their exact copies, but also to related sequences (Britten and Kohne, 1968). Therefore a saturation value may represent only the number of members of the family, and tells us nothing about the number of genes transcribed. Also, the kinetics are very slow for DNA-RNA hybridization to completion in an animal system, and unless an extremely high concentration of DNA and RNA are present, experiments must continue for weeks or months. During this time there is a danger of both DNA and RNA breakdown. Finally, there is difficulty in labelling RNA to a high enough specific activity to detect single copies or even tens of copies in the DNA genome.

Early experiments were usually performed with 'messenger RNA' from a heterogeneous tissue, such as liver or brain, or from HeLa cells or similar cell lines. Needless to say, such RNA contains at least a complex mixture of messengers, present at different concentrations, and perhaps many other RNA

components as well. Since the labelling and kinetics of hybridization of each component would be different, it is not surprising that few clear results were obtained from such work, apart from the conclusion that there were fewer RNA sequences present on the polysomes than in the nucleus (McCarthy, Shearer, and Church, 1969).

Attempts to avoid the problems of labelling *in vivo* by chemical means gave very high and totally improbable values (Williamson, Morrison, and Paul, 1970; de Jimenez *et al*, 1971). It now appears likely that the hybridization studied in these cases (using globin mRNA) was that due to the purine-rich non-coding sequences, rather than the actual 'messenger' portion of the molecule (Morrison, Paul, and Williamson, 1972). A far more accurate method of determining gene frequency is to perform hybridization under conditions of large DNA excess over long times using naturally labelled mRNA. This has been done for histone mRNA from sea urchins (Kedes and Birnstiel, 1971) and globin mRNA from duck reticulocytes (Bishop, Pemberton, and Baglioni, 1972). Neither mRNA is fully characterized, and in the case of the histone mRNA the evidence is circumstantial. The rapidly labelled fractions obtained from sea urchin embryos during development appear to hybridize to about 1000 copies of corresponding DNA, and in this case the hybrid is as near as can be determined 'perfect'; that is, it seems to represent true gene reduplication. In the case of duck, where the mRNA is better studied but less highly labelled, there are only very few copies of the hybridizing DNA, probably only 1 to 5 per haploid genome. This is true for DNA from both duck reticulocytes and erythrocytes, and while it may be argued that this rules out tissue differences, it is a pity data is not yet available for DNA from a non-erythroid tissue. The use of 'reverse transcriptase' to copy mRNAs at a very high specific activity should permit accurate DNA-RNA hybridization, giving reiteration frequencies for the available mRNAs in the near future, and then generalization concerning gene amplification can be made. If gene amplification is not a common phenomenon for mRNAs, a 'master gene' hypothesis such as that proposed by Callan (1967) loses much of its necessity.

Messenger RNA Synthesis and Processing

Since mRNA is synthesized on a DNA template, early attempts were made to find it in the nucleus. These were unsuccessful, and it was found that the most rapidly labelled RNA is a giant molecule, sedimenting at up to 100S and perhaps 50 times

larger than most cell mRNAs. This RNA species has been generally referred to as heterogeneous nuclear (Hn) RNA, and its relationship to mRNA is now becoming clear. All RNA synthesis in the nucleus appears to be into such giant RNA molecules. These nuclear RNA molecules not only are much longer than cytoplasmic mRNA, but also contain many more base sequences, as shown by DNA-RNA hybridization studies (Darnell *et al*, 1969; McCarthy *et al*, 1969). The nuclear HnRNA contains what appear to be mRNA sequences at its 3'-end, again from hybridization studies (Coutelle, Ryskov, and Georgiev, 1970). There have been several schemes suggesting that the excess, non-coding RNA in the large nuclear molecule is a transcribed control sequence, which may occur for many sequences and perhaps be related to the spectrums of different messengers that are switched on during differentiation (Britten and Davidson, 1969; Georgiev, 1969; Crick, 1971; Paul, 1972).

We have isolated HnRNA from embryonic mouse liver, an almost completely erythropoietic tissue which is quite similar to late erythroblasts in its cell composition. This HnRNA directs the synthesis of mouse globin when injected into *Xenopus* oocytes, at a much less efficient rate than purified polysomal mRNA, compatible with their being one mRNA sequence per HnRNA molecule (R. Williamson and C. Drewienkiewicz, unpublished results).

In the nucleus, after transcription of the HnRNA-containing mRNA sequences, an adenine-rich sequence is found on the 3'-end of the molecule. This may be added after transcription or may be part of the copied genome; it appears to be necessary for processing of the HnRNA from the nucleus to cytoplasm (Darnell *et al*, 1971). This type of adenine-rich sequence is also found in cytoplasmic mRNA (Lim and Canellakis, 1970; Edmonds, Vaughan, and Nakazato, 1971). It is sometimes referred to as a 'poly A' region but in fact contains up to 30% other nucleotides. It is not thought to occur in all mRNAs; those for histones in particular do not contain such a sequence (Schochetman and Perry, 1972).

When the mRNA is in the nucleus, it associates with a specific protein molecule called an 'informo-fer'. This protein does not appear to be messenger-specific; it has the same molecular weight, approximately 40,000, when isolated from different tissues (Samarina *et al*, 1968). In the cytoplasm the mRNA is associated with ribosomes, and when dissociated with chelating agents is complexed with two proteins, of molecular weight 130,000 and 68,000 (Lebleu *et al*, 1971). Neither of these is the same

as the infofer (Lukanidin, Georgiev, and Williamson, 1971). There is evidence of at least a measure of protein specificity for the mRNA in the polysome, since liver mRNP has a partially different protein spectrum from reticulocyte mRNP (Olsnes, 1971).

Medical Implications

It is worth considering the implications of the isolation of specific messenger RNAs on the perspectives for treatment of human diseases. The identification of the chromosomal loci of specific genes would provide direct evidence for linkage, supplementing data obtained from hereditary and diagnostic studies. It is now possible to visualize specific DNA-RNA hybrids formed *in situ*, if RNA or DNA can be obtained at a very high specific activity, several hundred thousand counts/min/ μ g. The chromosomal location of the sequences specifying ribosomal RNA in *Xenopus* and the location of 'satellite' DNA in mouse have been visualized using this technique, which is equally suitable for interphase nuclei or metaphase plates (John, Birnstiel, and Jones, 1969; Pardue and Gall, 1970). In view of the recent advances in intrachromosomal staining (O'Riordan *et al*, 1971), it should be possible to analyse the differences between normal and mutant cells directly by autoradiography of DNA-RNA hybrids with the relevant mRNA using stained metaphase plates.

There are several hereditary conditions characterized by the absence or reduced amount of a specific messenger RNA or its replacement by the product of a mutated gene; sickle-cell anaemia is the most prevalent, but some of the thalassaemias, the amino acidopathies, and many rare genetic diseases are also of this type. Although the defect is genetic, that is, localized in the base sequence of the DNA, it is possible that it might be corrected by the introduction of the correct mRNA, or of the gene corresponding to it. For when the correct mRNA has been isolated, it is possible to make a complementary copy of it using deoxynucleotides and animal virus 'reverse transcriptase' (Kacian *et al*, 1972; Ross *et al*, 1972; Verma *et al*, 1972). Such a DNA copy corresponds to the sense strand of the gene making the mRNA, and if it is an accurate and complete copy (which is not yet proven), it could be a genetic tool of extreme usefulness.

There are several possible ways in which such a mRNA or 'mDNA' molecule might be introduced into a human cell. Merrill, Geier, and Petricciani (1971) have reported that genes for α -D-galactose-1-phosphate uridyl transferase carried by a

transducing bacteriophage lambda are expressed when the virus 'infects' the human cell. Although there is no evidence that the cell is harmed by the presence of the phage, Merrill *et al* suggest that the virus does replicate in the human cells during 40 days exposure. The human cells used were galactosaemic human fibroblasts, which contain no GPU-transferase activity. In the galactosaemic cells exposed to phage, or to isolated phage DNA, the enzyme activity was detected. It is not clear whether the enzyme found was of bacterial or mammalian origin.

It is now possible to link large fragments of DNA to one another and it is likely that attempts will be made to attach 'mDNA' or mRNA to phage DNA and to infect human cells with the relevant human genes. There is also evidence that DNA molecules taken up from the medium can transform human cells in culture (Szybalska and Szybalski, 1962; Ayad and Fox, 1968). These represent alternative techniques for introducing specific genes or messengers into defective cells.

Whichever of these approaches proves to be successful, there is every prospect that the progress in isolation of mRNA should lead to direct attempts to correct genetic defects by supplementation with the accurate nucleotide sequence in the near future.

This work has been supported by grants to the Beatson Institute for Cancer Research from the Medical Research Council and the Cancer Research Campaign. I would thank Mr George Lanyon and Mrs Christine Drewienkiewicz for permission to use unpublished data, and Dr John Paul for helpful discussions.

REFERENCES

- Adams, J. M. and Cory, S. (1970). Untranslated nucleotide sequence at the 5'-end of R17 bacteriophage RNA. *Nature*, **227**, 570-574.
- Ayad, S. R. and Fox, M. (1968). DNA uptake by a mutant strain of lymphoma cells. *Nature*, **220**, 35-38.
- Bank, A. and Marks, P. A. (1971). Haemoglobin synthesis in thalassemia. *Series Haematologica*, Vol. 4, No. 3, 97-115.
- Benz, E. J. and Forget, B. G. (1971). Defect in messenger RNA for human hemoglobin synthesis in beta-thalassemia. *Journal of Clinical Investigation*, **50**, 2755-2760.
- Bishop, J. O., Pemberton, R., and Baglioni, C. (1972). Reiteration frequency of haemoglobin genes in the duck. *Nature New Biology*, **235**, 231-234.
- Blobel, G. (1971). Release, identification and isolation of messenger RNA from mammalian ribosomes. *Proceedings of the National Academy of Sciences of the United States of America*, **68**, 832-835.
- Borun, T. W., Scharff, M. D., and Robbins, E. (1967). Rapidly labeled, polyribosome-associated RNA having the properties of histone messenger. *Proceedings of the National Academy of Sciences of the United States of America*, **58**, 1977-1983.
- Britten, R. J. and Davidson, E. H. (1969). Gene regulation for higher cells: a theory. *Science*, **165**, 349-357.
- Britten, R. J. and Kohne, D. E. (1968). Repeated sequences in DNA. *Science*, **161**, 529-540.
- Burr, H. and Lingrel, J. B. (1971). Poly A sequences at the 3' termini of rabbit globin mRNAs. *Nature New Biology*, **233**, 41-43.
- Callan, H. G. (1967). The organization of genetic units in chromosomes. *Journal of Cell Science*, **2**, 1-7.
- Chantrenne, H., Burny, A., and Marbaix, G. (1967). The search for the messenger RNA of haemoglobin. *Progress in Nucleic Acid Research and Molecular Biology*, **7**, 173-194.
- Clayton, R. M., Truman, D. E. S., and Campbell, J. C. (1972). A method for direct assay of mRNA turnover for different crystallins in the chick lens. *Cell Differentiation*, **1**, 25-36.
- Coutelle, C., Ryskov, A. P., and Georgiev, G. P. (1970). Localization of messenger RNA near the 3'-end of the dRNA precursor molecule. *FEBS Letters*, **12**, 21-23.
- Crick, F. (1971). General model for the chromosomes of higher organisms. *Nature*, **234**, 25-27.
- Darnell, J. E., Maden, B. E. H., Soeiro, R., and Pagoulatos, G. (1969). The relationship of nuclear RNA to cytoplasmic RNA in HeLa cells. In *Problems in Biology: RNA in Development*, pp. 315-330, ed. by E. W. Hanly. University of Utah Press, Salt Lake City.
- Darnell, J. E., Philipson, L., Wall, R., and Adesnik, M. (1971). Polyadenylic acid sequences: role in conversion of nuclear RNA into messenger RNA. *Science*, **174**, 507-510.
- De Jimenez, E. S., Domínguez, J. L., Webb, F. H., and Bock, R. M. (1971). Comparative DNA-RNA hybridisation in differentiated cells. *Journal of Molecular Biology*, **61**, 59-71.
- Edmonds, M., Vaughan, M. H., and Nakazato, H. (1971). Polyadenylic acid sequences in the heterogeneous nuclear RNA and rapidly-labeled polyribosomal RNA of HeLa cells: possible evidence for a precursor relationship. *Proceedings of the National Academy of Sciences of the United States of America*, **68**, 1336-1340.
- Flatz, G., Kinderlerer, J. L., Kilmartin, J. V., and Lehmann, H. (1971). Haemoglobin Tak: a variant with additional residues at the end of the β -chains. *Lancet*, **1**, 732-733.
- Gaskill, P. and Kabat, D. (1971). Unexpectedly large size of globin messenger RNA. *Proceedings of the National Academy of Sciences of the United States of America*, **68**, 72-75.
- Georgiev, G. P. (1969). On the structural organization of operon and the regulation of RNA synthesis in animal cells. *Journal of Theoretical Biology*, **25**, 473-490.
- Gilmour, R. S. and Dixon, G. H. (1972). Biosynthesis of protamine in trout testis: V. Characterization of protamine mRNA in a trout liver cell free system. *Journal of Biological Chemistry*. (In press.)
- Gros, F., Hiatt, H., Gilbert, W., Kurland, C. G., Risebrough, R. W., and Watson, J. B. (1961). Unstable ribonucleic acid revealed by pulse labelling of *Escherichia coli*. *Nature*, **190**, 581-585.
- Heywood, S. M. (1970). Specificity of mRNA binding factor in eukaryotes. *Proceedings of the National Academy of Sciences of the United States of America*, **67**, 1782-1788.
- Hori, M. and Rabinovitz, M. (1968). Polyribosomal changes during inhibition of rabbit hemoglobin synthesis by an isoleucine antagonist. *Proceedings of the National Academy of Sciences of the United States of America*, **59**, 1349-1355.
- Jacob, F. and Monod, J. (1961). Genetic regulatory mechanisms in the synthesis of proteins. *Journal of Molecular Biology*, **3**, 318-356.
- John, H. A., Birnstiel, M. L., and Jones, K. W. (1969). RNA-DNA hybrids at the cytological level. *Nature*, **223**, 582-587.
- Kacian, D. L., Spiegelman, S., Bank, A., Terada, M., Metafora, S., Dow, L., and Marks, P. (1972). *In vitro* synthesis of DNA components of human genes for globins. *Nature New Biology*, **235**, 167-169.
- Kedes, L. H. and Birnstiel, M. L. (1971). Reiteration and clustering of DNA sequences complementary to histone messenger RNA. *Nature New Biology*, **230**, 165-169.
- Labrie, F. (1969). Isolation of an RNA with the properties of haemoglobin messenger. *Nature*, **221**, 1217-1222.
- Lane, C. D., Marbaix, G., and Gurdon, J. B. (1971). Rabbit haemoglobin synthesis in frog cells: the translation of reticulocyte 9S RNA in frog oocytes. *Journal of Molecular Biology*, **61**, 73-91.
- Lebleu, B., Marbaix, G., Huez, G., Temmerman, J., Burny, A. and Chantrenne, H. (1971). Characterisation of the messenger ribonucleoprotein released from reticulocyte polyribosomes by EDTA treatment. *European Journal of Biochemistry*, **19**, 264-269.
- Lim, L. and Canellakis, E. S. (1970). Adenine-rich polymer associated with rabbit reticulocyte messenger RNA. *Nature*, **227**, 710-712.
- Lingrel, J. B., Lockard, R. E., Jones, R. F., Burr, H. E., and Holder, J. W. (1971). Biologically active messenger-RNA for hemoglobin. *Series Haematologica*, Vol. 4, No. 3 37-69.

- Lockard, R. E. and Lingrel, J. B. (1969). The synthesis of mouse hemoglobin β -chains in a rabbit reticulocyte cell-free system programmed with mouse reticulocyte 9S RNA. *Biochemical and Biophysical Research Communications*, **37**, 204–212.
- Lockard, R. E. and Lingrel, J. B. (1971). Identification of mouse haemoglobin messenger RNA. *Nature New Biology*, **233**, 204–206.
- Lukanidin, E. M., Georgiev, G. P., and Williamson, R. (1971). A comparative study of the protein components of nuclear and polysomal messenger ribonucleoprotein. *FEBS Letters*, **19**, 152–156.
- Mathews, M. B., Osborn, M., Berns, A. J. M., and Bloemendal, H. (1972). Translation of two mRNAs from lens in a cell-free system from K12 ascites cells. *Nature New Biology*, **236**, 5–6.
- Mathews, M. B., Osborn, M., and Lingrel, J. B. (1971). Translation of globin messenger RNA in a heterologous cell-free system. *Nature New Biology*, **233**, 206–209.
- McCarthy, B. J., Shearer, R. W., and Church, R. B. (1969). The unstable nuclear RNA of mammalian cells. In *Problems in Biology: RNA in Development*, pp. 285–314, ed. by E. W. Hanly. University of Utah Press, Salt Lake City.
- Merril, C. R., Geier, M. R., and Petricciani, J. C. (1971). Bacterial virus gene expression in human cells. *Nature*, **233**, 398–400.
- Milner, P. F., Clegg, J. B., and Weatherall, D. J. (1971). Haemoglobin-H disease due to a unique haemoglobin variant with an elongated α -chain. *Lancet*, **1**, 729–732.
- Molloy, C. R., Sporn, M. B., Kelley, D. E., and Perry, R. P. (1972). Localisation of polyadenylic acid sequences in messenger RNA of mammalian cells. *Biochemistry*. (In press.)
- Morrison, M. R., Paul, J., and Williamson, R. (1972). The DNA-RNA hybridisation of ^3H -methylated mouse globin mRNA in conditions of RNA excess. *European Journal of Biochemistry*, **27**, 1–90.
- Nienhuis, A. W. and Anderson, W. F. (1971). Isolation and translation of hemoglobin messenger RNA from thalassemia, sickle cell anemia and normal human reticulocytes. *Journal of Clinical Investigation*, **50**, 2458–2460.
- Nienhuis, A. W., Laycock, D. G., and Anderson, W. F. (1971). Translation of rabbit hemoglobin messenger RNA by thalassaemic and non-thalassaemic ribosomes. *Nature New Biology*, **231**, 205–208.
- Olsnes, S. (1971). Further studies on the protein bound to the messenger RNA in mammalian polysomes. *European Journal of Biochemistry*, **23**, 557–563.
- O' Riordan, M. L., Robinson, J. A., Buckton, K. E., and Evans, H. J. (1971). Distinguishing between the chromosomes involved in Down's syndrome (trisomy 21) and chronic myeloid leukaemia (Ph^1) by fluorescence. *Nature*, **230**, 167–168.
- Pardue, M. L. and Gall, J. G. (1970). Chromosomal localisation of mouse satellite DNA. *Science*, **168**, 1356–1358.
- Paul, J. (1972). Chromosome structure and gene activation in eukaryotes: a general theory. *Nature*. (In press.)
- Pemberton, R. E., Houseman, D., Lodish, H. F., and Baglioni, C. (1972). Isolation of duck haemoglobin messenger RNA and its translation by rabbit reticulocyte cell free system. *Nature New Biology*, **235**, 99–102.
- Raff, R. A., Colot, H. V., Selvig, S. E., and Gross, P. R. (1972). Oogenetic origin of mRNA for embryonic synthesis of microtubule proteins. *Nature*, **235**, 211–214.
- Rhoads, R. E., McKnight, G. S., and Schimke, R. T. (1971). Synthesis of ovalbumin in a rabbit reticulocyte cell-free system programmed with hen oviduct RNA. *Journal of Biological Chemistry*, **246**, 7407–7410.
- Ross, J., Aviv, H., Scolnick, E., and Leder, P. (1972). *In vitro* synthesis of DNA complementary to purified rabbit globin mRNA. *Proceedings of the National Academy of Sciences of the United States of America*, **69**, 264–268.
- Samarina, O. P., Lukanidin, E. M., Molnar, J., and Georgiev, G. P. (1968). Structural organisation of nuclear complexes containing DNA-like RNA. *Journal of Molecular Biology*, **33**, 251–263.
- Schapira, G., Dreyfus, J. C., and Maleknia, N. (1968). The ambiguities in rabbit hemoglobin: evidence for a messenger RNA translated specifically into hemoglobin. *Biochemical and Biophysical Research Communications*, **32**, 558–561.
- Schochetman, G. and Perry, R. P. (1972). Early appearance of histone messenger RNA in polyribosomes of cultured L cells. *Journal of Molecular Biology*, **63**, 591–596.
- Stavnezer, J. and Huang, R. C. C. (1971). Synthesis of a mouse immunoglobulin light chain in a rabbit reticulocyte cell-free system. *Nature New Biology*, **230**, 172–176.
- Suzuki, Y. and Brown, D. D. (1972). Isolation and identification of the messenger RNA for silk fibroin from *Bombyx mori*. *Journal of Molecular Biology*, **63**, 409–429.
- Szybalska, E. H. and Szybalski, W. (1962). Genetics of human cell lines. IV: DNA-mediated heritable transformation of a biochemical trait. *Proceedings of the National Academy of Sciences of the United States of America*, **48**, 2026–2034.
- Verma, I. M., Temple, G. F., Fan, H., and Baltimore, D. (1972). *In vitro* synthesis of DNA complementary to rabbit reticulocyte 10S RNA. *Nature New Biology*, **235**, 163–167.
- Williamson, R., Clayton, R., and Truman, D. E. S. (1972). Isolation and identification of chick lens crystallin messenger RNA. *Biochemical and Biophysical Research Communications*, **46**, 1936–1943.
- Williamson, R., Lanyon, G., and Paul, J. (1969). Preferential degradation of messenger RNA in reticulocytes by ribonuclease treatment and sonication of polysomes. *Nature*, **223**, 628–630.
- Williamson, R. and Morrison, M. R. (1971). The isolation and DNA/RNA hybridization of messenger RNA for globin. *Series Haematologica*, Vol. 4, No. 3, 23–36.
- Williamson, R., Morrison, M., Lanyon, G., Eason, R., and Paul, J. (1971). Properties of mouse globin messenger RNA and its preparation in milligram quantities. *Biochemistry*, **10**, 3014–3021.
- Williamson, R., Morrison, M., and Paul, J. (1970). DNA-RNA hybridisation of 9S messenger RNA for mouse globin. *Biochemical and Biophysical Research Communications*, **40**, 740–745.