FUNDAMENTAL CANCER RESEARCH

THE nineteenth annual symposium on "Fundamental Cancer Research", sponsored by the M.D. Anderson Hospital and Tumour Institute of the University of Texas, was held at Houston, Texas, during March 4–6, 1965, the subject being "Development and Metabolic Control Mechanisms and Neoplasia". The sessions at the Symposium dealt with the following topics: "Biosynthesis and Control Mechanisms", "Molecular Basis of Early Development", "Molecular Basis of Later Development and Control" and "Comparative Studies of Control Mechanisms in Normal and Neoplastic Tissues". Several papers dealt with various aspects of the currently accepted sequence of events in biosynthesis, namely, DNA → RNA → protein, and especially aspects relating to development and differentiation.

R. M. S. Smellie (Glasgow) gave a review of the enzymatic synthesis of nucleic acids in bacteria and animal tissues, describing the various RNA and DNA polymerases involved. He pointed out that DNA is present in animal cells as a complex with protein, and that the mechanism by which it directs synthesis of new DNA remains to be determined. One function now generally assigned to histones is that of acting as gene repressors and they would accordingly play an important part in differentiation and development. Since different RNA fractions of the cell differ in their base composition, it is possible that the action of RNA polymerase is restricted to transcribing certain DNA regions in the nucleus. One approach to the study of these problems is by investigations on the synthesis of RNA in isolated nuclei and nucleoli the structural organization of which is maintained as intact as possible. R. B. Hurlbert (Houston), working with such systems, has presented evidence that the base composition of the newly formed labelled RNA can be altered by pretreatment of nuclei with different fractions of histone, or with trypsin to remove part of the histone originally present. The synthesis of the labelled RNA is inhibited by actinomycin *D*, indicating that the RNA is formed on a DNA template.

In the past few years a new approach has been developed in the study of the functions of hormones involved in development and growth. It is thought that their action is mediated through cellular mechanisms regulating nucleic acid and protein synthesis in their target tissues. It is possible that, in response to hormones, particular types of *m*RNA are formed; these then direct the coding of particular proteins, including enzymes which bring about specific developmental changes. This was discussed by J. R. Tata (London), who has shown that RNA polymerase of liver nuclei is increased by administration of thyroid and growth hormones. The effect is additive, indicating that each hormone enhances the formation of different fractions of RNA, including *r*RNA and *m*RNA. Also, incorporation of amino-acids into protein by mitochondria and microsomes is stimulated by thyroid hormone. Another example of the effect of thyroid hormone which was mentioned is the induced metamorphosis in the frog. The effects of other hormones involved in development were also discussed. In insect metamorphosis, the hormone ecdysone controls new differentiation processes by regulating the activity of specific genes involved in these processes (U. Clever, Lafayette, Ind.). A similar conclusion is arrived at also from the work of J. D. Wilson (Dallas, Texas) on the binding of [^{3}H]testosterone to chromatin fragments of nuclei from duck preen glands; the hormone binds to the fragments which are the major sites of synthesis of RNA in the nucleus. S. Cohen (Nashville, Tennessee) presented work on the isolation from mouse salivary gland of a protein of low molecular weight which stimulates epidermal growth. The stimulation of RNA and DNA synthesis in organ culture by this factor was also demonstrable.

Comparison of *m*RNA templates in rat liver and hepatomata were discussed by H. C. Pitot (Madison, W sconsin). Differences in the lifetime of templates for synthesis of enzymes concerned in amino-acid degradation were stated to occur in liver and hepatomata. H. G. Wittmann (Tübingen) reported work that is being carried out on the correlation between nucleotide changes in an *m*RNA and the resulting amino-acid replacements in the protein synthesized. This is done in tobacco mosaic virus RNA and its coat protein. An investigation of mutants of the virus obtained by treatment with mutagens has been carried out.

Investigations of the RNA and ribosome content of the unfertilized amphibian egg and embryo by D. D. Brown (Baltimore, Maryland) have shown that, while ribosomes are present mainly as monosomes in the unfertilized egg, polysomes are predominant in the early stages of the developing embryo, indicating that either more *m*RNA is formed or that its attachment to ribosomes is then initiated. Also the *s*RNA content of the egg is extremely small —possibly a further factor controlling protein synthesis. Modification of *s*RNA, which acts as an adaptor in protein synthesis on polysomes, may occur prior to differentiation, as shown by the work of N. Sueoka (Princeton, N.J.), who found that, as a result of infection of *Escherichia coli* with phage, a modified type of leucyl-*s*RNA is formed. S. Penman (New York) reported work on the relationship between protein synthesis and lipid metabolism in HeLa cells infected with polio virus. The polysomes of infected cells are found attached to lipoprotein membranes in contrast to those of normal cells. The infection is also accompanied by a stimulation of the lipid metabolism of the cell.

In a cell-free system *in vitro*, protein synthesis can be investigated by the rate of incorporation of labelled amino-acids; the presence of the necessary factors can thus be verified. The attachment of ribosomes to *m*RNA to form polysomes and the binding of aminoacyl-*s*RNA are prerequisites for polypeptide synthesis. The role of several factors involved in these processes, including enzymatic ones, has been discussed by R. S. Schweet (Lexington, Kentucky) and by H. Noll (Evanston, Illinois). The interchangeability of the various fractions from Novikoff tumour, rat liver and *E. coli* in such *in vitro* systems has been studied by A. C. Griffin (Houston). The components of the systems included amino-acid-activating synthetases, ribosomes, transfer enzyme fractions, *s*RNA and [^{14}C]aminoacyl-*s*RNA. It is interesting that, in general, a much greater interchangeability was observed between the components of tumour and liver than between those of *E. coli* and the mammalian tissues.

Aspects of metabolic regulations at the enzymatic and metabolic levels were discussed at the Symposium. Since the discovery, by Monod and his co-workers, of allosteric effects, many enzymes which show these properties have been described. Some enzymes exist in two configurations while others occur in sub-units which show reversible association and dissociation. A discussion of the characteristics, kinetics and behaviour of such enzymes towards substrates and inhibitors as compared with those of the more general type of enzyme was given

by C. Frieden (St. Louis, Missouri). Regulation of substrate concentration in the cell is another factor in control of biochemical reactions. Injection of creatine into chick embryo inhibits the endogenous formation of creatine by repressing the first enzyme involved in its biosynthesis. This inhibition is specific to creatine since its structural analogues are not effective (J. B. Walker, Houston). A similar negative feed-back control is found in the case of cholesterol synthesis by normal and regenerating liver. However, it is interesting that such a feed-back system is absent in all spontaneous and induced hepatomas tested (M. D. Siperstein, Dallas). Changes in the levels of substrates used as building-blocks in the synthesis of polysaccharides and in the activities of the enzymes concerned, and their correlation with different stages of differentiation of the slime mould, were discussed by B. E. Wright (Boston, Massachusetts). In another contribution, W. J. Rutter (Urbana, Illinois) discussed the two variants of fructose-diphosphate aldolase that have been found in animal tissues. During the development of the embryo there is a change-over from the predominance of one to that of the other. Two different metabolic roles are suggested for the two enzymes. Since the enzymes have distinct differences immunologically and in their fingerprint patterns, it is possible that they are coded by separate regions on the genome at different stages of cellular differentiation.

The Bertner Foundation Award for Outstanding Achievement in the Field of Cancer Research was presented to Prof. E. Chargaff (New York), who gave the Bertner Foundation Lecture at the Symposium on the topic: "On the Biological Consequences of Base-Pairing in Nucleic Acids". He discussed problems relating to the study of the primary structure of DNA. Although the regularities in the base composition of DNA apply to the whole DNA molecule, it is not known how far this is true of any given segment of the molecule. Several chemical methods for partial degradation of DNA have been developed. Clusters of nucleotides from the DNA chain can thus be obtained and identified and this may constitute a valuable approach to base sequence studies.

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POSSIBLE STRUCTURES FOR TRANSFER RIBONUCLEIC ACID: A TRIPLE-STRANDED MODEL

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ALTHOUGH considerable interest has been shown in the ribonucleic acid species called soluble, acceptor or transfer RNA (*t*RNA)[1] ever since its discovery[2,3], few concrete suggestions have appeared as to its structure. It is now believed from hydrodynamic measurements, X-ray investigations and electron micrographs[4] that *t*RNA is a rather rigid rod, although the evidence is not too convincing since these techniques are presently not refined enough to settle this point. Fresco *et al.*[5] first summarized the experimental evidence existing and brought forward two structures which are the essential basis of all models suggested to date. A chain bent back on itself in the form of a hairpin to form a helix, and a model with two hairpins joined together (V-model) were proposed. These authors believed that the helices in either model were interrupted to a varying degree by loops. A single completely helical (DNA-like) hairpin was also proposed[6-8]. Brown *et al.*[9] also envisaged the possibility that the hairpin and V model could be interchangeable. Sequence studies on unfractionated *t*RNA[10] led to a model only about 70 per cent helical where the bend was expanded to a large loop of about 15–20 nucleotides accommodating the anticodon and the rare bases. Since by definition *t*RNA has two distinct properties, that is accepting and transferring amino-acids, and since the phosphate backbone will provide little specificity for recognition, two non-helical regions should be necessary which might or might not be separated by helical regions. Novelli and others[11] suggested distinct acceptor and transfer sites, and recent work by Weill *et al.*[12] further supports the concept of two different operational sites, difficult to reconcile with the hairpin model, although evidence to the contrary has been presented too[13,14]. Spectral studies by Felsenfeld and Cantoni[15] point to a clustering of *A–U* and *G–C* pairs in serine specific *t*RNA, similar to previous findings of Fresco[16], who first reported two-step melting profiles for amino-acid specific *t*RNAs. From difference spectra studies on these purified *t*RNAs it was inferred[17] that each molecule contained two helical regions which melted separately and co-operatively. These data suggested that the ratio of *A–U* to *G–C* pairs was always considerably lower in the higher melting region.

All these reports have centred on a highly helical double-stranded structure of the Watson–Crick type, although no rigorous evidence has been presented so far for or against double strandedness in *t*RNA. In this article a triple-stranded model for *t*RNA will be offered to emphasize that other than double-stranded structures cannot be ruled out *a priori* at present. Evidence will be brought forward which will demonstrate the feasibility, advantages and disadvantages of such a structure as compared with various double helical models.

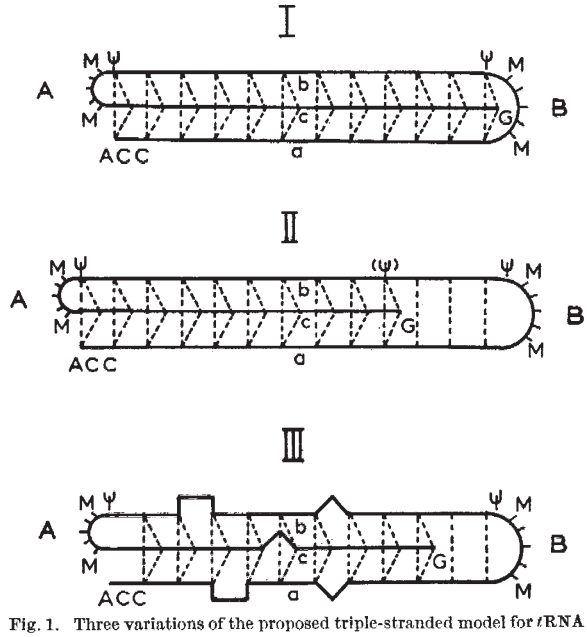

Fig. 1. Three variations of the proposed triple-stranded model for *t*RNA

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