Some of these represent a class of stable low-molecular-weight RNA in the nuclei of animal cells. There are differences in the weights of these in different species of mammal. The chloroplasts of higher plants also contain a range of low-molecular-weight RNA types, and a '5s' RNA that is distinct from the '5s' RNA of the cytoplasm.

(b) Ribosomal RNA tends to be degraded at specific points in the chain, leading to the formation of homogeneous low-molecular-weight fractions. These are not easy to distinguish from the RNA described above, and it is therefore important to establish that a low-molecular-weight component is not ribosomal. In general, the high resolution of gel electrophoresis, and the possibility of detecting trace amounts of RNA, has shown that it is almost impossible to prepare cell fractions in which degradation of RNA has been avoided. It is possible that part of the breakdown occurs *in vivo* in old ribosomes; a clear example of this in the case of chloroplast ribosomes will be shown.

(c) When the low-molecular-weight stable fractions and the ribosomal-RNA breakdown products can be identified or eliminated, it should be possible to detect messenger RNA. In the favourable cases in which a cell is synthesizing largely one or a few polypeptide chains, it should be possible to detect the messenger RNA by u.v. absorption, and its molecular weight determined by gel electrophoresis should correlate with the peptide chain length. The messenger RNA for haemoglobin from reticulocytes has been fairly well characterized. Attempts in this laboratory to isolate the messenger RNA for the light and heavy antibody chains will be described.

Primary Sequences and their Determination

By G. G. BROWNEE. (*Medical Research Council Laboratory of Molecular Biology, Cambridge*)

The methods that have recently been developed for determining the sequence of low-molecular-weight RNA will be reviewed. Emphasis will be placed on the relative simplicity of the radioactivity approach, which uses microgram quantities of nucleic acid, in contrast with methods relying on the detection of the ultraviolet absorption of the base. The fundamental approach in finding the sequence of a nucleic acid, which is the same whether the RNA is radioactive or not, will be illustrated by referring to the methods used to find the sequence of uniformly ^{32}P -labelled 5s RNA of *Escherichia coli*. The extent to which these radioactivity techniques are applicable to larger RNA molecules will depend, primarily, on the ease of the fractionation and on the yields that may be obtained of partial fragments, say 50–100 residues long, isolated from them. For many large RNA species it may be possible to isolate such

fragments from partial enzymic digests and by finding their sequence to reconstruct long stretches of the molecule.

The radioactivity approach is not limited to nucleic acid labelled *in vivo*, and attempts are now being made to find the sequence of fragments of non-radioactive RNA, which are labelled at their 5'-hydroxyl end with [^{32}P]phosphate *in vitro*. This may be achieved by using a specific virus-induced phosphokinase and [γ - ^{32}P]ATP. This may be the method of choice for RNA which cannot be conveniently labelled *in vivo*, such as mammalian RNA.

The sequence of 13 transfer RNA molecules is now known, about half determined by classical and half by radioactivity methods. In all cases an anticodon triplet has been located in a specific region of the molecule which is of opposite polarity and which forms base pairs with the predicted codon. A likely secondary-structure model can be predicted, both from the base sequence and from the fact that some regions of the molecule are particularly resistant to enzymic digestion. All transfer RNA species have some sequences in common, which could either represent common features necessary for the maintenance of the tertiary structure, or represent common functional regions. Attempts at correlating specific functions with specific regions of the molecule will be discussed by referring to results obtained with various transfer RNA molecules.

A Molecular Model for Transfer Ribonucleic Acid

By W. FULLER, S. ARNOTT and J. CREEK. [*Department of Biophysics and Medical Research Council Biophysics Research Unit, King's College (University of London), 26–29 Drury Lane, London W.C.2*]

Physical, chemical and biological studies on transfer RNA will be discussed in the context of a model that we have built for the three-dimensional structure of the molecule. With respect to its base-pairing this model is based on the clover-leaf structure. It has the T ψ C arm stacked between the amino acid and anticodon arms so that these three helical regions are coaxial. The T ψ C loop is assumed to have a conformation like that proposed by Fuller & Hodgson (1967) for the anticodon loop. The features of the structure (i.e. the dihydrouracil arm and the extra loop) that vary with the different transfer RNA species came close to each other in our model, forming a protuberance or knob on the helical stem formed by the amino acid, T ψ C and anticodon arms. The conformation of the stem is similar to that observed in crystalline fibres of extensive, regular, two-stranded RNA and is the

same for all transfer RNA species. We have packing arrangements for this model that allow us to account in a satisfactory way for the diffraction results from single crystals of transfer RNA that have been published. In addition to being stereochemically attractive the model accords well with current understanding of biological function.

Fuller, W. & Hodgson, A. (1967). *Nature, Lond.*, **215**, 817.

Biosynthesis and Maturation of Low-Molecular-Weight Ribonucleic Acid Species

By R. H. BURDON. (*Department of Biochemistry, University of Glasgow*)

t-RNA.* Since this small RNA species plays an important role not only in protein synthesis and its control but possibly also in the control of RNA synthesis itself (Maaløe & Kjeldgaard, 1966) it is essential to understand the processes involved in its own biosynthesis.

In cultured animal cells it has been demonstrated that a precursor to cytoplasmic *t*-RNA appears in the cytoplasm within a very short time of synthesis (Lal & Burdon, 1967; Burdon, Martin & Lal, 1967; Bernhardt & Darnell, 1969). This precursor, designated pre-*t*-RNA, is deficient in methyl bases, is somewhat longer than mature *t*-RNA (Burdon & Clason, 1969) and also has a more extended conformation (Burdon, 1967). It is postulated that, as part of a non-conservative maturation process giving rise to *t*-RNA, pre-*t*-RNA acts as substrate for the various specific *t*-RNA methylases that are located in the cell cytoplasm.

Removal of the essential amino acid L-methionine from the culture medium retards both the synthesis of pre-*t*-RNA and its maturation to *t*-RNA. It is thus possible that at least some of the pre-*t*-RNA bases require to be methylated for maturation to proceed and that the methyl-dependent step might be a nuclease attack at a specific site.

The low-molecular-weight RNA component of ribosomes (5s RNA). One molecule of this stable species (120 nucleotides long) is found in every larger ribosomal subunit in animal, plant and bacterial cells. In animal cells 5s RNA has also been detected within nucleolar particles contain the large ribosomal precursor RNA species (Warner & Soeiro, 1967). Although it has been shown in bacterial and in animal cells that 5s RNA and ribosomal RNA are synthesized at the same rate, it appears, however, that 5s RNA is not a part of the large 45s precursor of ribosomal RNA. Molecular hybridization has revealed that the nuclear DNA to which 5s RNA hybridizes can be separated from that to which ribosomal RNA hybridizes (Brown &

Dawid, 1968). Also, it has been demonstrated that the 28s RNA in a newly formed mammalian cell ribosome is not accompanied by a new 5s RNA (Knight & Darnell, 1967). Addition to the culture medium of the drug toyocamycin blocks the maturation in mammalian cells of the 45s ribosomal precursor RNA, but 5s RNA still appears in the cytoplasm (E. J. Smillie & R. H. Burdon, unpublished work).

A low-molecular-weight component from ribosomal RNA. When 28s RNA from the larger mammalian-cell ribosomal subunit is heated in the presence of detergent a small RNA molecule about 130 nucleotides in length is released (Pene, Knight & Darnell, 1968; Burdon & Clason, 1969). This small RNA can also be released by other treatments designed to break hydrogen bonds. Evidence has been obtained (Pene, Knight & Darnell, 1968) that this RNA arises from the same ribosomal RNA precursor molecules as does the 28s RNA. The function of this small RNA is unknown but it may play a role in maintaining the conformation of the RNA of the larger animal-cell ribosomal subunit. So far no equivalent RNA has been detected in bacteria.

Small monodisperse nuclear RNA from animal cells. Polyacrylamide-gel electrophoresis of RNA isolated from chromatin and nucleolar fractions from animal cells has revealed the presence of several distinct low-molecular-weight RNA species (Prestayko & Busch, 1968; Weinberg & Penman, 1968; Burdon & Clason, 1969). Their size ranges from 100 to 180 nucleotides, and they appear quite stable metabolically and are confined to the cell nucleus. It has been suggested (Prestayko & Busch, 1968) that these RNA molecules may be important in the regulation of genetic expression of chromosomes. Experiments with synchronized cultures of mammalian cells indicate that they are only synthesized at about the time of DNA synthesis in the S phase of the cell cycle (A. E. Clason & R. H. Burdon, unpublished work).

Bernhardt, D. & Darnell, J. E. (1969). *J. molec. Biol.* (in the Press).

Brown, D. D. & Dawid, I. B. (1968). *Science*, **160**, 272.

Burdon, R. H. (1967). *J. molec. Biol.* **30**, 571.

Burdon, R. H. & Clason, A. E. (1969). *J. molec. Biol.* **39**, 113.

Burdon, R. H., Martin, B. M. & Lal, B. M. (1967). *J. molec. Biol.* **28**, 357.

Knight, E. & Darnell, J. E. (1967). *J. molec. Biol.* **28**, 491.

Lal, B. M. & Burdon, R. H. (1967). *Nature, Lond.*, **213**, 1134.

Maaløe, O. & Kjeldgaard, N. O. (1966). *Control of Macromolecular Synthesis*. New York: W. A. Benjamin Inc.

Pene, J. J., Knight, E. & Darnell, J. E. (1968). *J. molec. Biol.* **33**, 609.

Prestayko, A. W. & Busch, H. (1968). *Biochim. biophys. Acta*, **169**, 339.

* Abbreviation- *t*-RNA, transfer RNA.