NUCLEIC ACIDS

More *t*RNA Fragments

from our Molecular Biology Correspondent

MANY properties of *t*RNA are curiously similar to those of enzymes. One is the ability of the molecule to re-assemble in a functional form from fragments produced by covalent cleavage, exactly in the manner of, for example, ribonuclease modified by subtilisin. It is known, for example, that the two fragments generated by cleavage in the anticodon loop will recombine to give a fully functional molecule in respect of acceptor activity. This was regarded as evidence against the suggestion that the anticodon site is involved in the recognition of the synthetase (though it must be admitted this hare was a poor runner from the outset). The argument has now, however, been taken a stage further by Hashimoto *et al.* (*Biochem. Biophys. Res. Commun.*, **37**, 777; 1969), who made similar fragments from yeast tyrosine *t*RNA, using T1 ribonuclease, and then chemically removed the 3′-terminal G residue of the 5′-fragment. In the absence of this nucleotide, the two halves still recombine, and indeed have higher acceptor activity than the native *t*RNA. This may be seen as proof that the anticodon is irrelevant to the synthetase recognition process.

It has now also been shown that the molecule can, with equally little consequence, be split in the dihydrouridine loop (Seno *et al.*, *Biochim. Biophys. Acta*, **190**, 285; 1969). Formylmethionine *t*RNA from *E. coli* has no G residues in the anticodon loop, and the T1 enzyme can therefore be used to break it at other points. Once again, recombination of the intact halves separated from such a digest leads to complete restoration of acceptor activity: the kinetics of aminoacylation show that both the Michaelis constant and the maximum rate are indistinguishable from those observed when the substrate is the intact *t*RNA.

Formylation of the methionyl-*t*RNA is also unimpeded. Moreover, when the fragments were separated after charging with methionine, the aminoacylated half by itself could not serve as substrate for the formylating enzyme. The recombined fragments retained, though in impaired degree, the ability to form initiator complex with ribosomes and the appropriate trinucleotide. At the same time, an anomalous (perhaps non-specific) binding showed itself with a polynucleotide containing initiator triplets, in the absence of ribosomes.

It has been noted (Imura *et al.*, *Nature*, **222**, 1147; 1969) that some acceptor activity is retained by yeast alanine *t*RNA even when large tracts of the sequence have been excised. Indeed, a mere vestige of the molecule, consisting of the two terminal segments, nine and fifteen residues long, was reported to show 3–5 per cent acceptor activity. It is, of course, necessary to exercise caution in interpreting such low levels of activity in terms of the nature of the active sites. With yeast valine *t*RNA, Mirzabekov *et al.* (*FEBS Lett.*, **4**, 281; 1969) were not able to obtain the same effect. But after cleavage at all three loops of the cloverleaf, involving the loss of a dinucleotide from one of them, four fragments could be isolated, which on mixing produced a species with 50 per cent acceptor activity.

This general approach should in time make it possible to delineate the elements of sequence and conformation involved in the various recognition processes, and the experiments lend themselves to all manner of variations. Mirzabekov *et al.* (*ibid.*, 218) have, for example, produced partially active hybrid species from the 3′-half of a *t*RNA from yeast, with the 5′-fragments from *E. coli* and rat liver.

MITOCHONDRIA

Membranes and Respiration

from a Correspondent

THE meeting of the Biochemical Society at the University of Warwick on November 13 and 14 was the first to be devoted to mitochondria. A colloquium on the biochemistry of mitochondria centred around the properties of the internal membranes of these organelles, and in particular on the structure, organization and function of the components that are involved in respiratory chain phosphorylation.

Dr J. B. Chappell (University of Bristol) described experiments that establish the interdependence of some of the anion-transporting systems present in the mitochondria of rat liver. Effectively a unidirectional cycle is set up with malate carried into the mitochondrion and oxoglutarate carried out, by means of specific carriers. Dr K. van Dam (Amsterdam) showed that several uncouplers behave in many ways like mitochondrial substrates, and that in addition to anion carriers the presence of relatively immobile positive charges (chiefly K^+) inside the mitochondrion means that it behaves in some respects like an anion-exchanger. Dr Chappell's paper and those by Dr E. Carafoli (Modena) on calcium ion transport and by Dr E. A. Munn (ARC Unit of Animal Physiology, Cambridge) on the electron microscopic appearance of mitochondria emphasized the different properties of mitochondria from various sources, of which various mammalian tissues, blowfly flight muscle, protozoa and yeasts seem to be the most popular choices.

Isolated mitochondria can bind Ca^{++} by three different but possibly interrelated processes: energy-linked, low-affinity and high-affinity binding. Energy-linked uptake of Ca^{++} occurs only in mitochondria from mammalian tissues and high-affinity binding only in some of these. A protein thought to be the carrier for high-affinity binding has been partially purified; it has a molecular weight of 100,000–200,000. Dr R. B. Beechey (Shell, Sittingbourne) has isolated a coupling factor with the properties of a protein previously recognized to confer oligomycin sensitivity (OSCP), with a view to testing his hypothesis that the activity of the dozen or so coupling factor preparations described so far is due to the presence of variable amounts of this protein in each preparation. He has shown that the activity of at least three of the coupling factors could be attributed to OSCP.

Dr P. Mitchell (Bodmin) provided experimental evidence to support his chemiosmotic hypothesis of respiratory chain phosphorylation, showing that protons are translocated across the inner membrane of rat liver mitochondria in numbers and at a rate sufficient to account for ATP synthesis. The protons are translocated outwards by intact membranes and digitonin fragments, and inwards by fragments produced by sonication (in keeping with the reversed polarity of these two types of fragment revealed by electron microscopy). Dr Mitchell went on to propose a scheme for the organization of the respiratory chain with