Studies on Thio-Bases in Rat Liver Transfer Ribonucleic Acid

By M. K. AGARWAL.* (*College of Physicians and Surgeons, Columbia University, New York, N.Y. 10032, U.S.A.*)

Out of nearly 30 'minor' bases, six sulphur-containing nucleosides have been reported in yeast and bacteria, and their presence is reported to regulate secondary structure in tRNA as well as rRNA in these organisms (Sueoka, Kano-Sueoka & Gartland, 1966). The studies summarized here were spurred because only presumptive evidence (Carbon, Hung & Jones, 1965) is available on this subject in mammalian systems and because such bases in tRNA could represent potentially susceptible sites for attack by various carcinogens (Agarwal & Weinstein, 1970).

Because of success with $\text{Na}_2^{35}\text{SO}_4$ in *Escherichia coli*, initially this material was injected four times in 100 μCi amounts (per 100g body weight) per injection. Most of the ethanol-precipitable radioactivity, isolated with the tRNA fraction, was soluble with ribonuclease and trichloroacetic acid. To assess the nature of the residual radioactive material, tRNA obtained from $\text{Na}_2^{35}\text{SO}_4$ -injected rats was submitted to electrophoresis on 7.5% polyacrylamide gels at pH 8.2, or sieved through Bio-Gel P-10 columns. In either case most of the radioactivity moved with a fraction lighter than 4S tRNA. The radioactive material, associated with tRNA after passage through the Bio-Gel P-10 column, was not eluted with 4S tRNA on subsequent fractionation of tRNA on 5–20% (w/v) sucrose density gradients. Similar results were obtained when either [^{35}S]cysteine or [^{35}S]methionine was used in place of $\text{Na}_2^{35}\text{SO}_4$.

Partial fractionation into individual tRNA species was carried out on BD-cellulose and RPC-Freon columns, but no enrichment of ^{35}S in selected fractions was observed. Also, aminoacylation followed by phenoxyacetylation, which selectively increases affinity of esterified tRNA on BD-cellulose columns, did not result in enrichment of ^{35}S in fractions containing the acylated derivative of tRNA. Similarly, prior feeding of the hepatocarcinogen acetamidofluorene, which increases the affinity of tRNA on BD-cellulose columns, did not alter the ^{35}S elution profile compared with the control profile.

Labelling of a few nucleotides may be masked by the bulk of the non-labelled bases. Paper electrophoresis of alkaline hydrolysates of tRNA did not reveal the presence of ^{35}S -containing nucleotides, nor did chromatography of such digests on urea-

DEAE-cellulose columns indicate the presence of 4-thiouracil derivatives.

Despite our extensive efforts, failure of ^{35}S label to be incorporated into mammalian tRNA may mean that an appropriate donor of thiol groups has still not been found. However, prior evidence (Carbon *et al.* 1965) indicating the possibility of thiolated bases in rabbit liver tRNA was based solely on rather non-specific activation of tRNA function. So the choice between the two alternatives seems clear.

Agarwal, M. K. & Weinstein, I. B. (1970). *Biochemistry, Easton*, 9, 503.

Carbon, J. A., Hung, L. & Jones, S. D. (1965). *Proc. natn. Acad. Sci. U.S.A.* 53, 145.

Sueoka, N., Kano-Sueoka, T. & Gartland, W. J. (1966). *Cold Spring Harb. Symp. quant. Biol.* 31, 571.

Size and Configuration of *Tetrahymena* Mitochondrial Deoxyribonucleic Acid

By R. A. FLAVELL and E. A. C. FOLLETT (introduced by I. G. JONES). (*Department of Biochemistry, University of Hull, Hull HU6 7RX, U.K., and Institute of Virology, University of Glasgow, Glasgow, W.2, U.K.*)

Mitochondrial DNA of animals and yeast is a circular duplex approx. 5 μm and 25 μm in circumference respectively (Borst & Kroon, 1969; Hollenberg, Borst, Thuring & Van Bruggen, 1969). In contrast Suyama & Miura (1968) found the mitochondrial DNA of *Tetrahymena pyriformis* ST to be linear and 17.6 μm long. The kinetic complexity of *T. pyriformis* T mitochondrial DNA has been shown to be $30 (\pm 5) \times 10^6$ daltons (Flavell & Jones, 1970), in good agreement with the estimate of Suyama & Miura (1968). Since this DNA appeared to be the only example of a genuine linear DNA other than certain viral DNA species we have reinvestigated the configuration of *T. pyriformis* T mitochondrial DNA and examined other possible models.

Mitochondrial DNA was prepared as described previously (Flavell & Jones, 1970) and purified by hydroxyapatite-column chromatography. In this way pure mitochondrial DNA was prepared within 12h of collection of the cells. Sedimentation of the DNA was studied in either 1M-sodium chloride or -caesium chloride ($\rho_{200} 1.397$), in 5mM-tris-HCl buffer, pH 7.5, by using the analytical band-centrifugation method (Vinograd, Bruner, Kent & Weigle, 1963).

Mitochondrial DNA from *T. pyriformis* T sedimented as a homogeneous band of $s_{20,w} 34\text{S}$. No components sedimenting faster than this were obtained, but a slower component (approx. 12S)

* Present address: Institut Pasteur, Paris, France.