Studies on Thio-Bases in Rat Liver Transfer Ribonucleic Acid

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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]cystine 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

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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)

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was present up to 50% of the material. Sedimentation in alkaline caesium chloride gave a sharp band at $s_{0, \text{obs.}}$ 20S with some trailing material, and denatured mitochondrial DNA sedimented at $s_{20, \text{w}}$ 97S in neutral solvent. These results are consistent with a linear DNA of approx. 30×10^6 daltons, without single-strand interruptions. Examination of the DNA in the electron microscope showed only linear filaments of $15 \mu\text{m}$, a size consistent with this and thus the same length as the genome, as previously determined (Flavell & Jones, 1970).

To test for cohesive ends, DNA ($10 \mu\text{g}/\text{ml}$) was heated to 70°C in 0.5M-sodium chloride, cooled slowly to 50°C and incubated for 3h, followed by sedimentation analysis. Annealed DNA sedimented at 33.8S and the control DNA (unincubated) sedimented at 35.0S. A circular DNA produced by self-annealing should have sedimented at approx. 38S. Hence no evidence for cohesive ends was obtained.

The possibility also remained that the DNA existed as circles which were broken by one double-stranded break on isolation. Renatured DNA should thus show circles in the electron microscope. The examination of this DNA is at present under investigation and will be discussed.

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Borst, P. & Kroon, A. M. (1969). *Int. Rev. Cytol.* **26**, 107.
Flavell, R. A. & Jones, I. G. (1970). *Biochem. J.* **116**, 811.

Hollenberg, C. P., Borst, P., Thuring, R. W. J. & Van Bruggen, E. F. J. (1969). *Biochim. biophys. Acta*, **186**, 417.

Suyama, Y. & Miura, K. (1968). *Proc. natn. Acad. Sci. U.S.A.* **60**, 235.

Vinograd, J., Bruner, R., Kent, R. & Weigle, J. (1963). *Proc. natn. Acad. Sci. U.S.A.* **49**, 902.

The Effect of Substitution of Deuterium Oxide for Water on Very Lysine-rich Histone-Deoxyribonucleic Acid Complexes

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The substitution of D_2O for water is known to result in elevated 'melting'-temperature values and therefore in correspondingly increased helical stability of synthetic polynucleotides and nucleic acids (for references see Lewin, 1967a,b). The enhanced stabilities are interpretable in terms of

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substitution of heavy-water bridges for ordinary water bridges and in terms of substitution of $-\text{O}-\text{D}\cdots\text{O}$ for $-\text{O}-\text{H}\cdots\text{O}$; the former bond is comparatively stronger than the latter (Lewin, 1969). The association of histones with DNA is also known to enhance the helical stability of DNA; this enhancement has been attributed to the histones bridging the two complementary DNA strands (for references see de Reuck & Knight, 1966). The association of histones with DNA via the ionic linkages involving the histone positively charged guanidinium (arginine) and ϵ -ammonium (lysine) groups has been computed to result in a significantly decreased extent of hydration directly involved in ionic linkages (Lewin, 1969, 1970); this applies also to displacement of water bridges as a result of direct hydrogen-bonding between the hydroxyl groups of the histone serine and threonine to DNA loci. Substitution of D_2O for water in aqueous solutions of histone-DNA would not be expected to result in increased helical stability if water bridges did not contribute effectively. We therefore investigated the variations in E_{255} of DNA and of lysine-rich histone F1-DNA associations with temperature, in both water and D_2O media. With a calf thymus histone F1-DNA ratio of 1:1, our results showed that at low ionic strength (1.5×10^{-3}) and pH 7 the curves were practically the same (and the resulting 'melting'-temperature value was $78.5 \pm 0.5^\circ\text{C}$). Simultaneous experiments under otherwise identical conditions confirmed the increase in 'melting' temperature of DNA on D_2O substitution for water (from $51.5 \pm 0.5^\circ\text{C}$ to $57.2 \pm 0.2^\circ\text{C}$).

Lewin, S. (1967a). *J. theor. Biol.* **17**, 181.

Lewin, S. (1967b). *Studia biophys.* **4**, 29.

Lewin, S. (1969). *Abstr. FEBS 6th Meet., Madrid*, p. 328.

Lewin, S. (1970). *J. theor. Biol.* **26**, 481.

de Reuck, A. V. S. & Knight, J. (1966). *Histones*.

London: J. and A. Churchill Ltd.

The Mechanism and Energetics of Histone Associations with Deoxyribonucleic Acid

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I have used correctly proportioned space-filling molecular models (Courtauld) and energetic considerations in evaluating the potential of association of histones with DNA. The selective removal of histones from chromatin requires increasingly higher electrolyte concentrations (for references see Busch, 1965), from which computations can be made as to the relative energy values