In the RC^{el} mutant the amounts of mRNA increased in the presence of trimethoprim.

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The Formation of MS Nucleotides in *Escherichia coli* Inhibited by Trimethoprim

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The unusual nucleotide MS I, guanosine tetraphosphate (Travers *et al.*, 1970), accumulates in RC^{tr} mutants of bacteria when required amino acids are removed (Cashel & Gallant, 1969), when levallophan is added (Boquet *et al.*, 1971) and when a mutant containing a temperature-sensitive aminoacyl-tRNA synthetase is incubated at a non-permissive temperature (Ehrlich *et al.*, 1971). It also accumulates in both RC^{tr} and RC^{el} strains when the bacteria are subjected to a 'stepdown' or diauxic lag in the growth medium (Harshman & Yamazaki, 1971; Winslow, 1971).

I have studied the formation of MS I nucleotide in RC^{tr} and RC^{el} strains of *Escherichia coli* inhibited with trimethoprim. In the presence of added exogenous purines and pyrimidines, RC^{tr} strains of *E. coli* accumulated MS I nucleotide to a concentration similar to that found in organisms deprived of required amino acids. In cultures not supplemented with purines or pyrimidines, the rate of accumulation of MS I nucleotide was lower, and reached lower values than when purines were supplied exogenously. In RC^{el} strains, MS I nucleotide concentrations did not increase, even when RNA accumulation was inhibited by purine deprivation.

If RC^{tr} strains were inhibited with both trimethoprim and rifampicin, MS I nucleotide concentrations rose rapidly and did not fall detectably over a further period of 20 min. In this, the cultures behaved like the temperature-sensitive valyl-tRNA synthetase RC^{tr} mutants at non-permissive temperatures (Ehrlich *et al.*, 1971), and not like amino acid-starved RC^{tr} strains (Wong & Nazar, 1970). Purine deprivation of RC^{tr} cultures did not affect the stability of the MS I fraction. The role of MS I nucleotide in the control of RNA synthesis was discussed.

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Effect of Pseudorabies Virus on the Metabolism of Low-Molecular-Weight Ribonucleic Acid in BHK-C13 Cells

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We have shown that shortly after infection of exponentially growing BHK-C13 cells with pseudorabies virus there is a gradual decrease in total RNA synthesis, and that this decline is mainly due to a large decrease in rRNA synthesis; similar results have been reported by Rakusanova *et al.* (1971). However, we have found that the synthesis of low-molecular-weight RNA species is less inhibited by pseudorabies infection than is rRNA synthesis.

Excellent separation of 4S and 5S RNA from the larger RNA species, and from each other, was obtained by electrophoresis on 10% polyacrylamide gels (Loening, 1967).

[5-³H]Uridine was used to label RNA in experiments, and RNA from cells labelled to equilibrium with [¹⁴C]uridine was used as internal control in all gels. After electrophoresis, the gels were sliced and solubilized (by a modification of the method of Tishler & Epstein, 1968) and their radioactivities counted by liquid-scintillation spectrometry.

When uninfected cells were labelled for 10 min the resulting cytoplasmic RNA showed a small 5S peak and a very small 4S peak. However, the bulk of radioactive material appeared between the 5S and 4S positions; this material appears to be precursor to tRNA (pre-tRNA) (Burdon, 1967; Bernhardt & Darnell, 1969; Shepherd, 1969). We have consistently found two peaks moving in the pre-tRNA position, and have designated the electrophoretic peak closer to 5S RNA as pre-tRNA I and the other as pre-tRNA II.

A longer label of uninfected cells (30 min or more) produced RNA that corresponded almost exactly to that of the internal 4S marker. However, after infection with pseudorabies virus the bulk of the material labelled for 30 min or longer remained in the pre-tRNA position on electrophoresis. During

a 'chase' of this labelled material, in the presence of actinomycin D and excess of unlabelled uridine, almost all of this 'pre-tRNA' moved to the 4S position. Incubation of this 'pre-tRNA' *in vitro* with extracts of infected or uninfected cells also caused this material to move to the 4S position. As with the RNA from uninfected cells, we obtained two peaks in the pre-tRNA position, and movement of the RNA appeared to be from peak I to peak II to 4S RNA. Incubation of infected cells with radioactive label for periods ranging from 10 to 75 min indicated that label first appeared in the pre-tRNA I position.

Unlike the larger rRNA species, 5S RNA production is apparently not appreciably inhibited by pseudorabies infection. At least part of this 5S RNA produced after infection appears not to be associated with ribosomes.

J. C. A. acknowledges with thanks the receipt of a grant from the Cancer Research Campaign.

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Specificity of Transfer Ribonucleic Acid Methylases from Normal Mouse Colon and 1,2-Dimethylhydrazine-Induced Colon Tumours

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Numerous reports of enhanced activity of tRNA methylases in neoplastic tissues have been published (summarized by Borek, 1971). Such increases could be due either to the stimulation of methylases present in the normal tissue or to the presence of additional methylases recognizing sites in the tRNA added as substrate not recognized by the enzymes present in normal tissue. This question can best be answered by direct studies of the nucleotide sequences containing the methylated bases formed *in vitro*, but the pattern of methylated products produced by crude mammalian tissue extracts acting on unfractionated tRNA is too complex for such analysis. The methylation of a number of highly purified tRNA species by extracts prepared from normal mouse colon and colon tumours has therefore been studied. Tumours were induced by 1,2-dimethylhydrazine, and extracts containing tRNA methylase activity were prepared and assayed as previously described (Pegg, 1971; Pegg & Hawks, 1971).

The following tRNA preparations from *Escherichia*

coli were found to be substrates for the formation of the methylated bases shown (in parentheses): glutamic acid tRNA (5-methylcytosine and 1-methyladenine), serine tRNA₃ (5-methylcytosine, 1-methyladenine, *N*²-methylguanine and *N*²*N*²-dimethylguanine) and formylmethionine tRNA (1-methyladenine, *N*²-methylguanine, 1-methylguanine and 5-methylcytosine). Purified yeast tRNA species (Gillam *et al.*, 1968) were also tested. Phenylalanine tRNA and arginine tRNA were not methylated, but aspartic acid tRNA (*N*²-methylguanine), alanine tRNA+threonine tRNA₁ (5-methylcytosine), threonine tRNA₂ (*N*²-methylguanine and 1-methyladenine), threonine tRNA₃ (*N*²-methylguanine and 1-methyladenine) and methionine tRNA₁ (5-methylcytosine) were substrates. Methylation was activated by Mg²⁺, NH₄⁺, putrescine or spermidine, and the cation giving maximal activity depended on the tRNA present as substrate. In general, NH₄⁺ or polyamine was required for the formation of methylated adenine and cytosine whereas methylated guanines were produced when only Mg²⁺ was present. Enzymes prepared from tumours produced products with each of the substrates tested similar to those found with extracts from normal colon, although the rate and extent of methylation were greater with the tumour extracts.

The sequences containing the methylated bases produced with formylmethionine tRNA and aspartic acid tRNA were determined by standard methods of enzymic degradation and fractionation of the oligonucleotides (Tener, 1967; Sanger & Brownlee, 1967). *N*²-Methylguanosine was present at residue 26 of aspartic acid tRNA in the sequence G-A-A-U-2MeG-G-G-C-. In formylmethionine tRNA *N*²-methylguanosine was present at residue 27 in the sequence -C-U-C-2MeG-U-C-, 1-methyladenosine was present at residue 59 in the sequence -T-ψ-C-A-1MeA-A-U-, 1-methylguanosine was present at residue 9 in the sequence -G-U-1MeG-G-A- and 5-methylcytidine was tentatively identified as present in position 49 in the sequence -G-7MeG-U-5MeC-G-. All of these sites in the tRNA structure are known to be occupied by methylated bases in some known tRNA sequences (Staehelin, 1971), and similar sites were methylated by both the tumour and the normal colon extracts.

This research was generously supported by the Michael Sobell Fellowship of the Cancer Research Campaign.

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