other purine, guanylic acid, the average rate of chain growth at 29° C is 26 nucleotides per second. At 37° C in the same medium the rate is 43 nucleotides per second, which is close to published estimates inferred from more indirect measurements of the rate of RNA synthesis, which range from about 13 to 90 nucleotides per second. The estimate by Stent's group provides an opportunity to ride the coupled transcription translation hypothesis; estimates of protein synthesis suggest that the rate of amino-acid polymerization is about 15 amino-acids a second at 37° C, almost exactly a third of the rate of RNA synthesis, and the code is of course a triplet code.

Stent's group has also shown, by repeating its experiment with bacteria growing at different rates, that the step time is correlated with the growth rate—the faster the growth rate the shorter the step time. The difference in step times is not, however, large enough to account for the difference in net rate of RNA synthesis. For example, in two bacterial cultures, one growing 3·2 times faster than the other, the average step times differ by only a factor of 1·7. This in turn implies that in the faster growing culture there are 4·6 times more nascent stable RNA molecules per unit of DNA template than in the slower growing cells. Bacteria must therefore have two mechanisms for varying their RNA content, one by altering the rate of synthesis, the other by altering the number of RNA molecules initiated.

After sifting the literature for estimates of the frequency of initiation and accepting published estimates of the proportion of the *E. coli* genome specifying stable RNA (all RNA except messenger), Manor *et al.* estimate that, in conditions of rapid growth, templates for stable RNA are completely covered by polymerase molecules.

MOLECULAR BIOLOGY

Forms of Transfer RNA

from our Molecular Biology Correspondent

THE operation of *t*RNA is governed by an extraordinary variety of chemical and conformational features of structure. It is not yet altogether clear which of these variants are biologically important and which are merely consequences of *in vitro* manipulations. A further layer of complexity has now been added by Yegian and Stent (*J. Mol. Biol.*, **39**, 45; 1969). This work starts from earlier observations on leucine-starved *E. coli*, which produced the unexpected result that the cessation of protein synthesis is not marked by the complete deacylation of the leucine *t*RNA. The experiment consists of determining the total acceptor activity after deacylation, and also measuring the residual activity after oxidizing the extracted RNA with periodate: this destroys the activity only of deacylated RNA, aminoacyl-*t*RNA being protected at the 3′-terminus. Yegian and Stent have now found that only about half of the *t*RNA releases leucine on alkaline deacylation; the rest contains a protecting group, resistance to alkaline hydrolysis, which is, however, displaced by leucine in the usual deacylating brew, by an enzymatic reaction. The resulting molecules behave normally in respect of alkaline deacylation and periodate oxidation, and are thus normal *t*RNA.

It is well known that there is more than one molecular species of leucine *t*RNA in *E. coli*, two peaks of leucine acceptor activity being easily resolved on chromatographic columns, and it turns out that the protected fraction occurs in only one of these (the second peak), whereas the residuum of leucyl-*t*RNA is all in the first. The notion that this species may fulfil some (obscure) regulatory part has been advanced by other workers, on the basis that, on the one hand, little of it is associated with the ribosomes during the exponential growth phase of the organism, and on the other hand, its proportion diminishes rapidly after infection with T2 phage. The nature of the protecting group has not been determined, but a suggestion is that it may be an N-acylated amino-acid, for these seem to confer considerable stability towards alkaline hydrolysis.

In a further paper (*ibid.*, 59) Yegian and Stent consider the aminoacylation of three species of isoleucine *t*RNA of *E. coli*, and find striking differences in that the reaction of one of them (species III) proceeds much more rapidly than that of the other two. These react very slowly indeed, especially at low magnesium concentration. It is shown that the three components are distinct molecular species, for they do not undergo mutual interconversion, but it seems likely that components I and II are in the low activity "denatured" state, described by Fresco, Sueoka and their associates. In the cell, however, it is fractions I and II which are substantially charged with their amino-acid, and III which is not. A curious observation is that species III, as isolated from leucine-starved cells, is largely inactive *in vitro* but becomes active after oxidation with periodate, though only when the 3′-terminus is protected by aminoacylation. The activation may well involve oxidation of a rare base—it is suggested of a thiol group. At the same time, Reid (*Biochem. Biophys. Res. Commun.*, **33**, 627; 1968) has reported that the reaction of ethyleneimine with thiol groups of *t*RNA causes inactivation towards particular amino-acids, which, however, do not include isoleucine. He suggests that this *t*RNA contains no thiolated bases, so that the problem evidently remains unresolved.

Three forms of tyrosine *t*RNA have also been isolated from *E. coli* infected with a transducing phage (Gefter and Russell, *J. Mol. Biol.*, **39**, 145; 1969). All three are aminoacylated at much the same rate, and are therefore not "denatured" forms. They differ grossly, however, in their ability to bind to the ribosome, and so participate in protein synthesis, and indeed one of them is essentially inactive in this respect. Oligonucleotide fingerprints show that the sequences are identical but that the three forms differ in one base modification next to the anticodon. The inactive form has an unmodified A residue in this position, whereas the other two have a large substituent group, one of them—the most active—with, the other without, a thiol group. Although not all the *t*RNA species of known sequence have modified bases in this position, it is not unreasonable to infer that in the present case this site is involved in attachment to the ribosome.

Yet another variant of *t*RNA is the dimeric form. The dimers of yeast alanine *t*RNA have now been isolated by Loehr and Keller (*Proc. US Nat. Acad. Sci.*, **61**, 1115; 1968). This dimer will accept two molecules of alanine. It has partial resistance to nuclease attack. An association which maintains the base-pairing scheme of the clover-leaf monomer can be devised.

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