

that addition of such templates causes a displacement in the association equilibrium in favour of a dissociated form. Thus with a ratio of nucleotide to enzyme of 0.4, a 19S component appears, and this is followed at higher ratios by one of 13S. At limiting nucleotide concentrations, only the last is present. Removal of the oligonucleotide by digestion with a nuclease reverses the reaction. These new components of the enzyme have been recognized before, and it has been supposed that the 19S and 24S states represent the dimer and trimer respectively of the 13S unit. The 13S form is evidently highly active, for it is observed that at elevated salt concentrations, at which the enzyme dissociates, its activity relative to a native DNA template is increased.

This latter observation is confirmed by So *et al.* (*Proc. US Nat. Acad. Sci.*, **58**, 1739; 1967), who have demonstrated enhanced activity in 0.2 M KCl solutions, using several DNA species as templates. Moreover, when the enzyme concentration is very high indeed, no such stimulation is elicited. So *et al.* suggest that under these circumstances all the initiation sites on the template DNA may already carry a polymerase molecule, so that an increase in the number of independent enzyme molecules through dissociation no longer has any effect. Alternatively, of course, the dissociation of the enzyme may be substantially less at such high concentrations; dissociation will in any case not be favoured by the low template to enzyme ratio.

It is also noted that the reaction at high salt is no longer subject to product inhibition, and so does not cease after a relatively short time, as it does at low salt concentrations. A further effect of the potassium chloride appears to be the suppression of non-specific RNA synthesis (polymerization without correct copying).

With denatured DNA it was observed that when the enzyme in 0.2 M KCl was added to the reaction mixture, addition of KCl now failed to produce the expected result. This is attributed to the instantaneous binding of the dissociated enzyme to the single strands; once bound, it is unable to aggregate again, so that no further disaggregating effect ensues on introduction of potassium chloride. If, on the other hand, the enzyme is added from a solution of low ionic strength, so that it enters the reaction mixture as 24S particles, the enhancement of activity by added potassium chloride is again observed. These results are not incompatible with those of Smith *et al.*: both high template to enzyme ratios and the added salt are factors which promote dissociation and activity.

So *et al.* note finally that different univalent cations vary in the degree in which they stimulate the polymerase activity. Whether this arises from differences in their efficacy in promoting dissociation or some other kind of action is not known.

## Dynamics of RNA Synthesis

from our Cell Biology Correspondent

SCHLESSINGER's group has shown that the ribosomal population *E. coli* consists of free 30S and 50S sub-units and polysomes without a pool of free 70S ribosomes, and as I mentioned two weeks ago (see *Nature*, **216**, 638; 1967), these observations agree with the latest ideas about chain initiation. In the current *J. Molecular Biology* (**29**, 395; 1967) Mangiarotti and Schlessinger

report further kinetic studies of the formation and lifetime of *mRNA*, ribosomal sub-units and polysomes which provide more evidence for many of the now almost conventional ideas about transcription and translation.

Mangiarotti and Schlessinger uniformly labelled all the stable RNA in a fragile strain of *E. coli*, which is easily lysed, with C<sup>14</sup> uracil and then switched the cells to a medium containing H<sup>3</sup> uracil. The ratio of H<sup>3</sup> labelled RNA to C<sup>14</sup> labelled RNA is then a measure of the ratio of new to old RNA molecules. Under their growth conditions complete chains of ribosomal RNA are synthesized in 1 to 2 minutes and it takes at least another 5 minutes to convert the ribosomal RNA into 30S and 50S sub-units by the addition of protein. They claim, unlike earlier workers, that it takes the same time to make either type of sub-unit. Not surprisingly, synthesis of ribosomes in *E. coli* is much quicker than in mammalian cells (see *Nature*, **214**, 963; 1967).

The specific activity of the label in free 30S and 50S sub-units and that in sub-units of the 70S monomers of polysomes increases at an identical rate. This means that there is rapid exchange between a pool of free sub-units and the sub-units of 70S ribosomes in polysomes. Furthermore, ribosomal RNA never appears in polysomes in an incomplete form. This is more evidence that ribosomal RNA does not act as *mRNA* (see *Nature*, **214**, 963; 1967). It also means that the attachment of ribosomes is not necessary for the release of ribosomal RNA from its DNA template.

About 30 per cent of the total H<sup>3</sup> uracil incorporated within 6 minutes of giving the label, in other words before new ribosomal sub-units have been completed, is in *mRNA*. Virtually all the *mRNA*—about 3 per cent of the total cellular RNA—detectable at any time is in polysomes. These results confirm other evidence that translation begins before transcription has been completed and moreover it shows the *mRNA* molecules must be functional for almost all their lifetime, which on average is 11 to 12 minutes. Under different conditions Forchhammer and Kjeldgaard (*J. Mol. Biol.*, **24**, 459; 1967) found that about half the *mRNA* in a mutant strain of *E. coli* has a half-life of about 5 to 7 minutes whereas the other half was relatively stable with a half-life of between 42 and 70 minutes. Clearly the stability of *mRNA* in *E. coli* is very dependent on the strain and growth conditions.

In eucells *mRNA* is generally more stable than in bacteria, but even so in several situations its stability depends on whether or not the cells are dividing; stability increases as the cells lose their mitotic activity. Stewart and Papaconstantinou have just reported an interesting example of this (*J. Mol. Biol.*, **29**, 357; 1967). Bovine lens epithelial cells synthesize three closely related proteins,  $\alpha$ ,  $\beta$  and  $\gamma$  crystallin. Actinomycin D completely inhibits crystallin synthesis in embryonic cells but not in adult cells, although in both cases all RNA synthesis is inhibited. The embryonic cells actively divide whereas the mitotic index of the adult cells is low. Stewart and Papaconstantinou infer from this that as epithelial cells age the *mRNA* molecules which specify crystallin become stabilized and this stabilization may well be related to the decrease in mitotic activity of the ageing cells, but how it is effected is a mystery.