the melting temperatures could be measured as a function of chain length.

In the first place, it was found that complexes were formed only with the template DNA, or that from the closely related species—T2 with T4, T3 with T7, the extent of homology being somewhat lower in the latter pair. Second, in standard conditions nothing smaller than a decamer was retained by the DNA, but above this the stability of the complexes rose rapidly with length. Moreover, the T-odd species, with a higher GC content (about 65 per cent as against 50 per cent in the T-even) formed complexes more stable by 15°–20° C in the length range 12–17. The base-compositions of the fragments in each case fell within a narrow range, so that the effect of chain length alone could be determined. The onset of complex formation occurs at chain lengths of 10 and 12 respectively in the two series, but in the presence of magnesium, octamers become the shortest species to generate detectable complexes.

Although differences will be expected in situations involving longer polymers, also containing non-complementary sequences, these results should help to provide a quantitative basis for interpreting hybridization experiments. DNA–DNA complexes will be a little less stable, RNA–RNA rather more. It may be remarked how much greater the minimum stable chain length is than in homopolymer systems, such as oligo-G–poly-C. It has long been supposed that in the latter there is a gain in stability from the alignment of the oligomers on the polymer, so that there is in effect a duplex with multiple single-strand breaks.

An example of hybrid formation with poor complementarity is given by Saunders *et al.* (*Biochemistry*, **8**, 1222; 1969). They have searched for clusters of like bases in a phage DNA (TP-84, which grows in strains of a thermophilic bacterium) by complexing with polyhomoribonucleotides. The procedure, developed by Szybalski and his co-workers, involves the determination of buoyant densities in a caesium chloride gradient, these being markedly changed on formation of complexes, when the polymer, which can be in large excess, is added to the system. Saunders *et al.* separated the strands of the DNA, and examined them individually for complex formation. The light strand was found to bind poly-U, and apparently also poly-C, though here the change was less convincing. The results suggest the presence of purine runs in the DNA, but the converse association—that of the heavy strand with poly-A or poly-G—could not be demonstrated. Saunders *et al.* suggest that the reason is the inaccessibility of parts of the denatured chain. It may be worth considering, however, that poly-U is the only homopolymer which is not appreciably stacked at room temperature; consequently it is the only one for which the uncomplexed state is not stabilized by stacking energy, so that it would not be surprising (especially because the deoxy-chain has a much smaller stacking propensity) to find that poly-U forms a more stable duplex than poly-A. A comparison of the results of Saunders *et al.* with those of Niyogi suggests either that there are runs of considerable length of a single base in the DNA, or that the buoyant density method detects very weak complexes (which indeed might be expected), or again that the system is stabilized by many shorter complementary stretches, separated by short looped-out segments; such an arrangement would also be expected to have enhanced stability.

PROTEIN SYNTHESIS

What Initiation Factors Recognize

from our Cell Biology Correspondent

IDEAS about the role of the initiation factors which are required to initiate protein synthesis in bacterial cell-free systems will have to be revised in the light of a report in *Nature* (**222**, 750; 1969) by Rudland, Whybrow, Marcker and Clark. They argue convincingly that the initiation factors recognize some unique region of the initiating fMet-tRNA_f as well as the formyl group itself. This means, of course, that the initiation factors are specific for fMet-tRNA_f and do not recognize any aminoacyl-tRNA with a blocked amino group.

In a cell-free system, two codons, AUG and GUG, specify the binding of fMet-tRNA_f to 70S and 30S ribosomes and this binding is stimulated by GTP and initiation factors. But what do the factors recognize—the blocked amino group of the fMet-tRNA_f or something more specific? Lucas-Lenard and Lipmann suggested that the factors recognized a blocked amino group and therefore any aminoacyl-tRNA with a blocked amino group could act as a chain initiator. Their experiments had shown that initiation factors stimulate the binding of N-acetylPhe-tRNA to ribosomes programmed with poly-U.

Rudland *et al.* have reinvestigated this reaction and have also tested the stimulation of binding of fLys, fTyr and fVal-tRNAs and fMet-tRNA_f and fMet-tRNA_m by the initiation factors. They find that apart from the natural initiator, fMet-tRNA_f, only fPhe-tRNA binds in significant amounts to 70S or 30S ribosomes in the presence of the initiation factors. Moreover, binding of fPhe-tRNA is more than ten times less than binding of fMet-tRNA_f. A control experiment excludes the possibility that any of the tRNAs are damaged during the chemical formylation reaction. Thus, contrary to the prediction of Lucas-Lenard and Lipmann, any aminoacyl-tRNA with a blocked amino group is not sufficient for factor stimulated initiation. The factors are highly specific, capable even of differentiating between tRNA_f^{Met} and tRNA_m^{Met}, the nucleotide sequences of which were found to be so similar that they do not suggest what structural differences distinguish natural initiator tRNA from other tRNAs.

But if the initiation factors are so specific for fMet-tRNA_f why does any fPhe-tRNA or acetylPhe-tRNA bind to ribosomes in the presence of the factors? Rudland *et al.* offer the only possible suggestion, namely, that the structures of fPhe-tRNA and fMet-tRNA_f are similar enough to allow the initiation factors partially to recognize the former as well as the latter.

Although the initiation factors recognize some unique structure in the tRNA_f-Met molecule, the formyl group is obligatory for initiation. If the methionyl-tRNA_f is not formylated it does not bind to ribosomes in the presence of all the other components of the reaction mixture. This may imply that formylation induces a change in the configuration of the initiator tRNA_f-Met, especially because it is known that acylation of other aminoacyl-tRNAs alters their tertiary structure. It may be that the factors need to recognize simultaneously the formyl group and the unique structure of the tRNA_f-Met. The answer will have to wait until someone obtains crystals of tRNA_f^{Met} large enough for X-ray crystallography to reveal the unique structure that the initiation factors recognize.