NEWS AND VIEWS

Where Ribosomes begin Working

THE initiation process in protein synthesis consists of several steps. First, the messenger is bound to the smaller of the ribosomal subparticles together with at least three initiation factors, formylmethionyl transfer RNA and GTP. Protein synthesis can begin when this complex is joined by the 50*S* ribosomal subparticle. Until about a year ago, it was supposed that the ribosome starts to translate a messenger by binding to the 5′ end and then working its way along to the first AUG or GUG codon, when construction of a protein molecule would begin. There are, however, several lines of evidence to show that ribosomes need not start at the end of a messenger. Protein synthesis can probably be initiated simultaneously at several sites on a polycistronic messenger (a messenger coding for several proteins) and it is also known that circular messenger molecules can direct protein synthesis without ambiguity. This is a serious dilemma, for AUG and GUG are codons for amino-acids other than formylmethionine that occur elsewhere in the messenger while the other combinations may turn up by means of a simple displacement of the reading frame of the triplet code. How then does the ribosome know where to begin ?

In this issue of *Nature*, Dr Joan Argetsinger Steitz from Cambridge (page 957) and Drs J. Hindley and D. Staples from Bristol (page 964) describe the isolation and nucleotide sequence determination of those segments of a messenger RNA (isolated from viral RNA molecules) to which the *E. coli* ribosome binds in the presence of formylmethionyl-transfer RNA. In both cases, highly radioactive viral RNA (isolated from either R17 or Qβ phage, the host of which is *E. coli*) was bound to ribosomes in conditions appropriate for the initiation of protein synthesis. The excess viral RNA lying outside these complexes was removed with pancreatic ribonuclease, and the fragments of RNA protected by the ribosomes were isolated.

The R17 phage RNA codes for three proteins: the phage coat protein, a maturation protein and an RNA polymerase. Dr Argetsinger Steitz has isolated three RNA fragments, each about thirty-five nucleotides long, and has determined their sequences using the procedures devised by Sanger and his colleagues. These fragments have been identified as arising from the initiation regions of the three genes coded by the R17 phage RNA. For example, one of her fragments contains a sequence AUG.GCU.UCU.AAC.UUU which clearly codes for the known N-terminal sequence of the coat protein which begins with formylmethionine. alanine.serine.asparagine.phenylalanine. . . . Her other two fragments were similarly identified. Drs Hindley and Staples have identified with Qβ fragment (with a sequence which was fortunately such that no partial product analysis was necessary) as the beginning of the Qβ coat protein gene. There are two hard conclusions to be drawn from these sequences. First, formylmethionyl transfer RNA is coded in these four segments by AUG. Second, in no case are these AUGs preceded directly by a termination codon, which could be any one of the codons UAG, UAA or UGA.

But what does the ribosome detect that is different in these AUGs from other AUGs ? The first question, to which there is no firm answer, is whether the ribosome can detect other AUGs—perhaps they are all buried in helical regions of secondary structure, as, for example, found in part of the sequence corresponding to the R17 coat protein by Adams, Jeppesen, Sanger and Barrell (*Nature*, **223**, 1009; 1969). In other words, it is just conceivable that the only AUGs exposed to the ribosomes in the native RNA are the initiator AUGs, and it may then be significant that the AUG of the R17 coat initiation site seems to be exposed at the end of a short helix. At the Cold Spring Harbor Symposium, at which Dr Argetsinger Steitz first presented two of her sequences (*Nature*, **223**, 133; 1969), it seemed that secondary structure might well point to the answer.

Ribosomes may also locate these AUGs by means of a special sequence adjacent to each initiator codon, so that the real sequence signifying initiation would be somewhat longer than the simple AUG. This notion is attractive because three of the four fragments contain a sequence UUUGA two or three residues away from the starter AUGs. Unfortunately, not merely is there nothing similar in the fourth sequence, but there is another reason why this sequence does not seem to be the whole answer. The R17 RNA has three binding sites when ribosomes and initiation factors from *E. coli* are used. But as Lodish found (*Nature*, **224**, 867; 1969), when ribosomes and initiation factors from *B. stearothermophilus* are used, only the A protein initiation site is seen; both the coat and A protein initiation fragments contain this UUUGA sequence—yet only one of them is recognized by the *B. stearothermophilus* ribosome.

There are more complicated ways in which these initiation sites could be recognized, but at present nobody has a convincing solution to the problem. It may turn out that there are several initiation factors, specific for different beginnings of genes—at present that seems the most likely explanation. In the meantime, there may be a further windfall from the isolation of the three R17 initiation sites. The order of the three genes has never been properly determined, partly because it is impossible to do genetic mapping of the R17 phage. Knowing these sequences and that determined by Adams *et al.*, a group including Drs Jeppesen and Argetsinger Steitz has been able to locate the three genes on two large RNA fragments isolated by Drs R. Gesteland and P. Spahr: it seems that the gene order is: A protein–coat protein–synthetase.

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