Dual Purpose Factor

ONE of the ideas which has persisted among molecular biologists for a long time in spite of little evidence is that some control over gene expression might be exerted at the level of translation of messenger RNAs into protein. Although control at the level of transcription of the genome into RNA is, of course, well established, there have been few data or, indeed, theoretical reasons, to support the notion that translation might also be regulated. But more recently, the reluctance to abandon this other possible control system has been justified, for in the past year or so several groups have reported evidence which suggests that factor F3, one of the proteins required for the initiation of protein synthesis in *Escherichia coli*, may have a role in determining which messenger RNAs a ribosome can recognize.

The first real indication that this might be the case came from experiments which Hsu and Weiss reported in 1969. They found that crude preparations of initiation factor F3 extracted from *E. coli* cells infected with phage T4 promoted the formation of an initiation complex between T4 messenger RNA and *E. coli* ribosomes and assisted the subsequent translation of the messenger. This factor from infected cells did not, however, promote the initiation of translation of *E. coli* messengers or RNA phages. By contrast, crude preparations of F3 factor from normal uninfected cells supported the translation of *E. coli* messengers and phage RNA as well as T4 messengers. And, of course, it has been repeatedly observed that F3 factor is essential for the initiation of translation of natural messengers *in vitro* but is not essential for the translation of synthetic messengers in the cell-free system.

These observations, confirmed and extended by other groups, led to the growth of the idea that different species of F3 factors may exist, each species programming a ribosome to recognize some particular set of initiation sequences and therefore set of messengers. The translation of a messenger, once it had been synthesized, would depend on the presence of the appropriate F3 factor, without which translation could not begin. Clearly the chief prediction of this model of translational regulation is simply that more than one species of F3 factor exists in *E. coli*.

Earlier this year in *Nature New Biology* (**234**, 44 ; 1971) Berissi, Groner and Revel reported that a homogeneous F3 factor, known as F3–B2, isolated from normal *E. coli*, is required for the recognition by the 30S ribosome of the specific initiation sites on native MS2 phage RNA. If the MS2 RNA is denatured ribosomes bind to it in the absence of F3–B2 but not at the three specific initiation sites. Revel's group also briefly mentioned that they had succeeded in isolating a second species of initiation factor, F3–B4, from uninfected *E. coli* which does not allow the recognition of the initiation sites of MS2 RNA but does promote the binding of ribosomes to phage T4 messengers. On page 236 of this issue of *Nature New Biology*, Lee-Huang and Ochoa report in full similar findings. They have isolated from normal *E. coli* two F3 factors, one of which promotes the recognition of MS2 initiation sites while the other promotes the recognition of T4 messenger sites. They also find that after *E. coli* cells have been infected with T4 phage the latter factor can still be isolated but the former cannot, presumably because it is somehow inactivated during the infection.

These data and those of Revel and his colleagues seem to establish that in *E. coli* there are at least two species of F3 factor which differ in their specificity and which can be conveniently assayed with MS2 and T4 phage RNAs. Whether yet more species of F3 factor exist remains an open question but the fact that there are two factors justifies further searches for other species with different specificities. If they can be found it will be hard to avoid the conclusion that translation is regulated at the stage of chain initiation.

Messenger recognition and selection may be a function of F3 factor but as Sabol and Ochoa report (see page 233) that is not the sole function of this sort of factor. Their experiments are consonant with the idea that F3 factor plays a crucial role in dissociating 70S ribosomes that are released from a messenger RNA at the end of a round of protein synthesis. Having tagged F3 factor with ^{35}S, they measured the extent of its binding to washed 30S and 50S ribosomal subunits and washed 70S ribosomes and found that only the first of these three sorts of particle binds the factor at a molar ratio of unity. Furthermore, they have shown that by incubating 70S ribosomes with F3 factor the ribosomes can be dissociated into a 50S subunit and a 30S subunit with one molecule of factor bound, and when, by raising the concentration of Mg^{2+} ions, 50S and 30S ribosomal subunits are caused to associate to yield a 70S ribosome, the molecule of F3 factor is displaced from the 30S subunit.

These data, together with many other published observations that a factor is required to dissociate 70S ribosomes running off a messenger and that free 30S ribosomal subunits have associated initiation factors, lead Sabol and Ochoa to support the model, championed by Davis and his colleagues, which implicates F3 factor in ribosome dissociation. They envisage that a 30S ribosomal subunit carrying a bound molecule of F3 recognizes an initiation sequence in a messenger and binds to it. When the 50S subunit joins this initiation complex to form the 70S ribosome the molecule of F3 is displaced and finds itself free to bind to the 30S subunit of a 70S ribosome running off a messenger at the end of translation. This binding dissociates the 70S ribosome and the 30S subunit replete with F3 factor is ready to initiate another round of translation. The amount and the specificity of available F3 factor in a cell may well therefore determine which messengers can be translated and what proportion of a cell's ribosomes are to be found as free 70S ribosomes and 30S and 50S subunits.