PROTEIN SYNTHESIS

Translation not Universal

from a Correspondent

NEW information is still being obtained about various aspects of the translation process, during which genetic information carried on messenger RNAs is used in the biosynthesis of specific protein molecules. It is becoming increasingly clear that in spite of the universal nature of the genetic code, different parts of the translation apparatus are not entirely interchangeable between different organisms. In the past, various workers have synthesized the globin portion of rabbit haemoglobin *in vitro*, using with gay abandon unwashed ribosomes from rabbit reticulocytes (carrying the messenger RNAs for the globin chains and the factors necessary for protein synthesis) together with aminoacyl tRNAs from *Escherichia coli* or yeast. Because the primary structures of the globin chains are known and the system has several technical advantages, it has proved particularly useful for exploring the degeneracy of the genetic code.

But, in a careful study, Hunter and Jackson (*Europ. J. Biochem.*, **15**, 381; 1970) have re-examined the products actually obtained when globin is synthesized in this way, and sadly conclude that substantial levels of miscoding occur during translation in this heterologous system. They demonstrated this by using tRNA charged with radioactive amino-acids, astutely choosing three amino-acids (cysteine, methionine and valine) which occur rather rarely in the globin molecule, so as to be able to study where these were incorporated within the protein. They mixed the radioactive globin which was synthesized with carrier amounts of natural rabbit globin, and fingerprinted tryptic digests of the mixtures. The fingerprints were stained with ninhydrin to reveal the peptides arising from the carrier material and then autographed to find out which peptides contained the radioactive amino-acids.

In all cases, they found that substantial amounts of the labelled amino-acids occurred in peptides not normally expected to contain them. In some instances peptides with altered mobilities were produced by the amino-acid changes. In a few surprising cases they found that peptides which ought to have contained labelled amino-acids did not, and they were unable to explain this entirely satisfactorily although they wondered if some residual reticulocyte tRNAs from the unwashed ribosomes were competing very effectively for these sites. A judicious control experiment demonstrated that, under identical conditions, incorporation from reticulocyte valine tRNA was found only in the true globin peptides. From their results they were able to deduce that the cysteine tRNA (which normally recognizes UG^{U}_{C}) was also probably reading the arginine codons CG^{U}_{C} because of mismatching of the codon–anticodon complex. Hence in five cases cysteine was introduced in place of arginine. They also suggested that both the methionine $tRNA_F$ and $tRNA_M$ were extensively reading GUG, and that methionine was being introduced instead of valine into various points in the molecule. Hunter and Jackson believe that the most probable explanation of these somewhat traumatic results is that the reticulocyte ribosomes are unable to position all the *E. coli* tRNA species correctly relative to the codons on the mRNAs, presumably because of nucleotide sequence differences between the tRNAs from *E. coli* and reticulocytes.

Some important results from the wonderful world of factors are reported by Revel and his colleagues in two recent papers (*FEBS Lett.*, **9**, 213 and 218; 1970). The attachment of mRNAs to ribosomes in *E. coli* is known to depend on the presence of three protein factors A(F1), B(F3) and C(F2). Factor B is necessary for the recognition of natural mRNA templates, as opposed to synthetic polynucleotides. They find that factor B is heterogeneous, and can be split into multiple components by chromatography on columns of DEAE-cellulose and hydroxylapatite. These fractions are distinguished from each other by preferentially stimulating the translation of different mRNAs *in vitro*. Thus one of the fractions stimulates the translation of bacteriophage MS2 RNA, while the other activates the translation of bacteriophage T4 mRNA (assayed by the product of translation of the lysozyme cistron). The two activities were not entirely separated from each other, and it is unclear whether this is because of incomplete purification, or whether each really possesses some activity in the other system. The fraction stimulating the translation of MS2 RNA could be further split into at least three peaks. They elegantly demonstrated that one of these peaks caused preferential translation of the coat protein cistron. Further evidence as to the regulatory role of the B factor fractions was obtained by studying cells infected with bacteriophage T4. After infection, the stimulatory activity of factor B for the translation of T4 mRNA, and particularly the late messenger, was considerably increased, while factor B activity in MS2 translation was diminished. These findings suggest that a further mechanism governing the control of gene expression is operating at the translational level. It will be important news indeed if analogous systems are characterized in eukaryotic cells.

PROTEIN SYNTHESIS

Jumping on the Bandwagon

from a Special Correspondent

IF the meeting on mammalian protein synthesis held at Cold Spring Harbor last week had been held six months ago, almost all of the thirty-three contributions would have been dramatic news. But research has progressed so fast that by now, however, the topics discussed seem hackneyed and weary; as repetitive report succeeded report, it seemed at times that the predominant view of participants was that the only problem worth solving in molecular biology is how haemoglobin synthesis is initiated in reticulocytes.

Haemoglobin turns out to be initiated in a way very similar to bacterial proteins; as D. Wilson (Cornell University), R. J. Jackson and A. R. Hunter (University of Cambridge), A. Yoshida (University of Washington) and H. F. Lodish *et al.* (MIT) showed, a special initiator tRNA ($tRNA_f^{met}$) provides a methionine residue to start the globin chain. This methionine is cleaved from the protein when the growing chain is about twenty amino-acids long, perhaps because it is then no longer protected by the ribosomes and

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