Regulation of Protein Synthesis

from a Correspondent

At a Biochemical Society colloquium on the regulation of protein biosynthesis in eukaryotic cells, held in London on February 7, Dr R. J. Jackson (University of Cambridge) started the meeting by mentioning that met-tRNA$_f$ binds to native 40S subunits before mRNA in rabbit reticulocyte lysates. This binding is abolished by lack of haemin, double-stranded RNA and oxidized glutathione, and, as theory would predict, these agents all cause a non-specific inhibition of protein synthesis. The binding of mRNA is prevented by aurinetricarboxylic acid, and the final step in the formation of the initiation complex, the binding of the native 60S subunit, is prevented by edeine. It may well be that the first step is the one which is normally subject to control in cells when they need a generalized shutdown of protein synthesis, as, for example, during mitosis, under conditions of amino-acid deprivation or at high temperature.

The following speaker, Dr R. H. Stevens (National Institute for Medical Research), described what seems to be a very specific kind of regulation of protein synthesis. When mouse myeloma 5563 cells are incubated at low temperature (20–25° C) they continue to synthesize heavy and light chains of immunoglobulin IgG, but fail to secrete them. When cells which have been incubated at low temperature are returned to 37° C they immediately resume secretion, but appear to suppress the synthesis of heavy chains. Dr Stevens believes that this is because IgG suppresses heavy chain synthesis, and showed that IgG injected into *Xenopus* oocytes along with myeloma mRNA can lower the ratio of light to heavy chains synthesized in this system. He also found that IgG binds specifically to heavy chain mRNA at physiological salt concentrations. These data seem irreproachable ; yet, as Dr Stevens himself pointed out, it is hard to see how the IgG could get at the mRNA because it is usually supposed to be separated by a membrane.

Dr H. Bloemendahl (Catholic University, Nijmegen) reviewed his group's work on the translation of calf-lens crystalline mRNA, which is progressing well along familiar lines. Both the 10S and 14S mRNA contain an A-rich segment, and by using oligo(dT) columns the group have found that only a very small fraction of the RNA isolated from gradients is active—that which binds to the dT cellulose column. The mRNA can be copied into DNA with reverse transcriptase primed with either oligo(dT) or oligo(dG). The significance of the latter observation is unclear; in either case the copy seems to be incomplete. There is still no explanation for the discrepancy between the size of the mRNA and the size of the proteins they code for; hopefully the new hybridization experiments with anti-mRNA will help solve the mystery of the extra stretches. The problem of poly(A) regions came up again when Drs J. B. Lingrel (Cincinnati, and at present at MRC Molecular Biology Unit, Cambridge) and J. Mansbridge (Glasgow, and shortly at University of Queensland, Brisbane) disagreed about the length of the poly(A) segment in globin mRNA. The Cincinnati group claim a length of either six or seven residues, whereas the Glasgow workers find fifty-three and seventy-seven. It seems to be impossible to decide who is right on internal evidence alone, and perhaps they will exchange materials and techniques to resolve the issue. Neither the speakers nor any member of the large audience was prepared to guess what the poly(A) is for, if it is there.

Professor H. Arnstein (King's College, London) talked about the evidence for and the significance of "free" mRNA. He has found that in the reticulocyte most of the free mRNA is for α chains, which is consistent with the finding that α chains are made on smaller polysomes than β chains—one would expect some mRNA not to have ribosomes on it as a result of purely statistical considerations. Two kinds of stochastic models for the process devised by Professor P. J. Higgins of the mathematics department of King's College, however, both failed to square precisely with the experimental data. It seems possible that some interesting control mechanisms may be at work.

To end the morning session, Professor P. N. Campbell (University of Leeds) reviewed a spectacular example of a hormonal control on protein synthesis—the transformation from the pregnant mammary gland to the lactating mammary gland. Preliminary data suggest that the pregnant gland does not contain any mRNA for lactalbumin, the predominant product of the lactating gland. Another case of a hormonal switch was described by Dr M. J. Clemens (NIMR) who has studied the induction of yolk protein synthesis by livers of male toads treated with oestradiol, but in spite of the large changes in polysome distribution and activity induced by these hormone treatments, the basic mechanisms are still far from clear.

CYTOKININS

Mechanism of Action

from our Plant Biochemistry Correspondent

Cytokinins are plant hormones which regulate cell division, and all of those known are purine derivatives, principally of adenine. Much interest has been centred on the possibility that cytokinins might regulate cellular processes through the modification of specific transfer RNAs, because cytokinins have been found adjacent to the anticodon in several plant tRNAs. This hypothesis, never very clearly spelled out mechanistically, has recently been criticized on quantitative grounds and another, quite different hypothesis seems to be emerging.

The quantitative limits of cytokinin incorporation into tRNA have been found by Elliott and Murray (*Biochem. J.*, **130**, 1157 ; 1972) to be far too low to allow a role for this process in the regulatory action of cytokinins. They estimated the absolute incorporation of labelled 6-benzyladenine (6-BA) into the tRNA of soybean callus cultured on 6-BA for 6 days, and found no significant incorporation of the intact molecule. Some incorporation of label by transbenzylation occurred, but only to the extent of one molecule of cytokinin per 755–2,210 molecules of tRNA. Clearly, this very low degree of incorporation cannot account for the regulatory effects of cytokinins unless only a very specific small fraction of the tRNA is implicated.

Other lines of evidence seem to indicate that cytokinins might be acting in some way analogous to cyclic AMP in mammalian cells. Wood and Braun (*Proc. US Nat. Acad. Sci.*, **70**, 447 ; 1973) have shown that a derivative of cyclic AMP, 8-bromoadenosine-3′,5′-cyclic monophosphate, completely replaces cytokinin in the growth of tobacco callus. The derivative is much more resistant to the plant phosphodiesterases than is cyclic AMP itself, and this might account for the smaller effects observed with cyclic AMP. In another study, Ralph, McCombs, Tener and Wojcik (*Biochem. J.*, **130**, 901 ; 1972) have found that cytokinins can modify the protein phosphorylation capacity of plant tissues and extracts. Kinetin stimulated the rates of protein phosphorylation in floated leaf disks of Chinese cabbage, and inhibited phosphorylation in nuclei/chloroplast extracts both from Chinese cabbage and other plants. Purified ribosomes also exhibited protein kinase activity which was inhibited by cytokinins. Both groups of workers draw parallels between the cytokinins and cyclic AMP in relation to the manifold regulatory effects of both classes of compounds.