We postulate that a membrane-associated control factor exists that can have an oxidized or a reduced form. The results show that GTP is also involved. The control factor, X, may have to exist in a GTP complex, X-GTP, in order for protein synthesis to occur. This complex is readily obtained if factor X is reduced, but can be derived from oxidized factor X only in response to high GTP concentrations. The ratio of oxidized factor X to reduced factor X present is thus postulated as being critical. According to this model, the normal liver has a greater ratio than the regenerating one. The results from the diamide experiments, in which oxidation occurs, agree with this model, and lend strong support to its validity.

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The Site and Nature of Transferase II-Guanosine Triphosphate Complex Attachment to Mammalian Ribosomes

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In mammalian protein synthesis, transferase I binds the incoming aminoacyl-tRNA to the acceptor site on the ribosome. This is followed by peptide-bond formation at the acceptor site, and the peptidyl-tRNA is then translocated to the donor site by the action of transferase II. This enzyme requires GTP and its action is inhibited by cycloheximide. We are now able to add detail to the mechanisms involved.

With the use of either Millipore filters or Sephadex columns, complex-formation between [¹⁴C]GTP and purified transferase II has been demonstrated. This reaction is not impeded by cycloheximide. Both the isolated complex and a mixture of GTP and enzyme have been used to study the binding of this complex to the ribosome and its subsequent fate.

As shown by using labelled GTP, binding of the enzyme-nucleotide complex occurred very rapidly when the peptidyl-tRNA was in the donor position, but less extensively when the peptidyl-tRNA was in the acceptor position. With prolonged incubation the latter type of ribosomes eventually bound

amounts comparable with those bound by the donor type; reaction with puromycin demonstrated that this was due to transfer of the peptidyl-tRNA to the donor site during prolonged incubation. It was concluded that the presence of peptidyl-tRNA at the acceptor site occludes binding of the complex.

When cycloheximide was added to the reaction mixture it decreased the amount of labelled GTP bound. When GTP was first bound to the ribosomes in the presence of transferase II, and cycloheximide was subsequently added, the bound GTP was expelled in a form that passed through a Millipore filter and was thus no longer attached to the enzyme. It was concluded that the association of GTP with the enzyme is altered on binding and becomes susceptible to the action of the inhibitor.

Studies are presently being undertaken with labelled transferase II to determine whether cycloheximide similarly decreases the amount of enzyme bound and whether addition of the inhibitor to ribosomes already bearing labelled transferase II causes release of the enzyme at the same time as the GTP is expelled.

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Biosynthesis of Tryptophanase by Polyribosomes and by Ribonucleic Acid and Ribosomes Isolated from *Escherichia coli*

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We have previously reported (Parish, Khairul Bashar & Brown, 1971) the biosynthesis of tryptophanase (EC 4.2.1.e) by using polyribosomes isolated from induced cultures of *Escherichia coli* M.R.E. 600. We now report the synthesis of a protein with the same chromatographic properties as tryptophanase by using RNA from induced cells and an S-30 fraction from repressed cells. The properties of proteins synthesized by the polyribosome system and the RNA-S-30 fraction system are compared.

RNA was isolated from induced cells by the method of Parish (1968) and 'preincubated S-30 fraction' was isolated from repressed cells by the method of Nathans (1968). Mixtures of RNA, S-30 fraction, cofactors necessary for protein synthesis, 19 unlabelled amino acids and one radioactive amino acid were incubated at 37°C. The incorporation of [³H]leucine into a protein that co-chromatographed with tryptophanase on DEAE-cellulose was demonstrated. The incorporation was shown

to be time-dependent, to be inhibited by chloramphenicol and to be energy-dependent. Another control experiment established that RNA from repressed cells was totally inactive for this synthesis. The activity of RNA from the induced cells was largely associated with the 23S fraction of rRNA when RNA was isolated from sucrose density gradients before use in the assay.

The *N*-terminal amino acid of tryptophanase is methionine and the *C*-terminal amino acid is valine (Morino & Snell, 1967). Incubations with either [³⁵S]methionine or [³H]valine were employed to establish whether these amino acids are terminal in the product synthesized *in vitro*. It was established that methionine is the *N*-terminal amino acid in tryptophanase synthesized in the RNA-S-30 fraction system, but not in tryptophanase synthesized in the polyribosome system; conversely it was found that the *C*-terminal valine was not present in the tryptophanase synthesized in the RNA-S-30 fraction system but was present in that synthesized in the polyribosome system.

Radioactive tryptic-peptide 'maps' obtained from protein synthesized with either system (with labelling with either [¹⁴C]leucine or [³⁵S]methionine) were superimposable with the 'map' obtained from purified tryptophanase except that for the protein obtained from the RNA-S-30 fraction incubation ten of the 32 tryptic peptides were very weak. We conclude that essentially complete tryptophanase subunits are synthesized in both systems, but that the polyribosome system does not initiate protein synthesis and that the RNA-S-30 fraction system produces molecules with incomplete *C*-termini. Nevertheless the chromatographic analysis forms a satisfactory assay for tryptophanase mRNA.

The properties of the protein synthesized in the RNA-S-30 fraction system were examined in greater detail to establish whether the incomplete *C*-termini affected the tertiary or the quaternary structure. By using material purified in the absence of tryptophanase carrier we showed that the newly synthesized protein did bind the cofactor (pyridoxal phosphate), but then when analysed by sucrose-density-gradient centrifugation it sedimented largely as subunits.

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Lincomycin as a Chloroplast Probe

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Chloroplasts contain a protein-synthesizing machinery involving 70S ribosomes, but it is not clear which proteins are made on these ribosomes (Kirk, 1970). Studies with chloramphenicol have given conflicting results, probably because of the lack of specificity of action of this compound in some plant tissues (Ellis, 1969). One of the similarities between isolated chloroplast and bacterial ribosomes is that protein synthesis is inhibited by lincomycin; protein synthesis by cytoplasmic ribosomes on the other hand is not inhibited (Ellis, 1970). Lincomycin is a highly effective inhibitor of chloroplast development in greening pea apices (Ellis & Hartley, 1971) and can be used to determine the sites of synthesis of chloroplast proteins in this tissue.

When etiolated pea apices are detached and illuminated with white light for 48h, several enzymes increase in specific activity as follows: ribose phosphate isomerase, threefold; phosphoribulokinase, 15-fold; ribulose diphosphate carboxylase, sixfold; phosphoglycerate kinase, twofold; NADP-specific triose phosphate dehydrogenase, sixfold; RNA polymerase, threefold. The specific activities of NAD-specific triose phosphate dehydrogenase and succinate dehydrogenase are unaffected by illumination of the apices. The rate of incorporation of [¹⁴C]leucine into protein by plastid pellets increases eightfold on the basis of fresh weight and threefold on the basis of weight of protein; it has previously been shown by polyacrylamide-gel electrophoresis that chloroplast ribosomal RNA components increase in amount on illumination of the apices (Ellis & Hartley, 1971).

When the apices are illuminated in the presence of 5 μ M-lincomycin all the enzymes except ribulose diphosphate carboxylase show similar increases in specific activity as the controls, even though chlorophyll synthesis is depressed by 75%. The increase in activity of the carboxylase is inhibited by 50% by 1 μ M-lincomycin. The increase in the leucine-incorporating ability of plastid pellets is totally prevented by 5 μ M-lincomycin.

These results suggest that chloroplast ribosomes synthesize some component of ribulose diphosphate carboxylase and at least some of the chloroplast ribosomal proteins. The other enzymes appear to be synthesized in the cytoplasm, even though some of them are located exclusively in the chloroplasts and are involved in photosynthesis. These results support the conclusion derived from genetic studies that chloroplasts are not autonomous (Kirk & Tilney-Bassett, 1967).