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