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Studies on an Initiation Factor for Mammalian Protein Synthesis

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A protein fraction necessary for polyphenylalanine synthesis at low Mg^{++} concentrations has been isolated both from crude rat liver ribosomes and from the 100 000 x g supernatant of rat liver homogenates. Washed ribosomes without this protein fraction synthesize polyphe at higher Mg^{++} concentrations (> 8 mM) only. It has been shown that the factor is involved in the initial phase of polyphe synthesis and that it is not identical with the elongation factors transferase I and II. The factor stimulates the incorporation of phenylalanine into the N-terminal position of polyphe as well as the formation of N-acetyl-phenylalanyl-puromycin at low Mg^{++} concentrations. The binding of N-acetyl-phe-tRNA to the ribosomal 40 S subunit is stimulated by the factor in dependence on GTP and dithiotreitol. The factor activity can be destroyed completely by treatment with N-ethylmaleimide. The factor was partially purified by gel filtration on Sephadex G200 and chromatography on DEAE cellulose.

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The Role of Met-tRNA$_M^{Met}$ and Met-tRNA$_F^{Met}$ in the Elongation of Polypeptide Chains by Mouse Liver Polysomes.

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Two methionine accepting tRNA species corresponding to tRNA$_F^{Met}$ and tRNA$_M^{Met}$ from mouse ascites tumor cells were tested for their ability to donate methionine into internal positions of growing polypeptide chains on mouse liver polysomes. Both tRNA species can function in the elongation of polypeptide chains as judged by their ability to incorporate methionine into protein in the absence of chain initiation. The insertion of methionine into internal positions was confirmed by Edman degradation and by cyanogen bromide cleavage of the radioactive products synthesized in vitro. When both tRNA^{Met} species were present in saturating concentrations in the cell-free system a strong preference for the incorporation of methionine from Met-tRNA$_M^{Met}$ became apparent. In a series of double-label experiments saturating concentrations of ^{3}H-Val-tRNA^{Val} were introduced into the cell-free system together with increasing amounts of ^{35}S-Met-tRNA$_M^{Met}$ or ^{35}S-Met-tRNA$_F^{Met}$. While there appeared to be no competition between Val-tRNA^{Val} and Met-tRNA$_M^{Met}$, the level of methionine incorporation was depressed by about 30 % when Met-tRNA$_F^{Met}$ was the only donor of methionine and Val-tRNA^{Val} was present in saturating amounts. Our results demonstrate that Met-tRNA$_F^{Met}$ can serve as a donor of methionine in peptide chain elongation. The fact that Val-tRNA^{Val} and Met-tRNA$_F^{Met}$ can compete with each other in peptide chain elongation indicates that a certain degree of miscoding may be involved in the incorporation of methionine from the latter tRNA species.

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INTERMEDIATE REACTIONS IN THE BINDING OF AMINOACYL-tRNA TO RAT LIVER RIBOSOME.

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Purified transferase I (TF I) from rat liver bind readily aa-(^{32}P)tRNA or (^{14}C)phe-tRNA to form a relatively stable complex (A). This reaction requires Mg^{++} and is inhibited by GTP. The product is retained on nitrocellulose filters. A purification procedure was developed for the preparation of complex A free of unbound aa-tRNA using DEAE cellulose filters. Deacylated tRNA does not combine with TF I to form complex A. TF I forms an apparently transient and highly unstable complex (B) with (^{3}H) and (^{32}P)GTP. This reaction also requires Mg^{++} but at higher concentrations than the synthesis of complex A. Binding of GTP to TF I is significantly enhanced in the presence of aa-tRNA. Only during the formation of complex C (i.e. TF I--aa-tRNA - GTP) the TF I shows a rather low GTPase activity but not during the synthesis of complex B. Complex C is very unstable alone, but becomes rapidly bound to ribosome even at 0° (complex D). During this step most GTP is split to GDP. Complex D may be isolated by ultracentrifugation or by gel filtration on Sephadex G-100. The latter method may be also used for the separation of complex A but not of B or C that are decomposed during the chromatography. Results reported in this paper indicate that the mechanism of aa-tRNA binding to ribosome in mammalian systems is different from that in bacterial cells. The primary reaction involved in peptide elongation in mammalian cells is apparently the formation of a TF I--aa-tRNA complex and not the combination of the binding enzyme with GTP as demonstrated for bacterial systems. These differences may help to explain the relatively high species-specificity of peptide elongation factors.

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INHIBITION OF POLYPHENYLALANINE SYNTHESIS BY A tRNA FRACTION IN A WHEAT EMBRYO SYSTEM.

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We have studied the effect of transfer RNA different from tRNA^{phe} on poly-U directed polyphenylalanine synthesis in a cell free peptide synthetizing system from wheat embryos. The ribosomes and the supernatant fraction were prepared according to the procedure of J.E. Allende and M. Bravo (J. Biol. Chem., 241 (1966) 5813); under small modifications the system was completely dependent on tRNA added. The tRNA^{phe} used was purified from wheat embryos and accepted 1,200 picomoles per 1 O.D. unit at 260 nm. The total tRNA from wheat embryos was chromatographed on a benzoylated DEAE cellulose column; the fractions obtained were checked in the system in quantities equimolar to the purified tRNA^{phe} used; only one of the fractions was shown to inhibit the polyphenylalanine synthesis. The inhibition appears to be competitive. The inhibitory fraction from BD cellulose was rechromatographed on a reverse phase column; the inhibitory activity was eluted in a position different from that of the tRNA^{phe} lacking CCA.