MINIREVIEW

Eukaryotic Polypeptide Elongation System and Its Sensitivity to the Inhibitory Substances of Plant Origin (43988)

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Abstract. The structural and functional characteristics of the elongation system (ribosomes and elongation factors) are presented. The immunochemical and diagnostic meaning of the ribosome investigations is considered. Evidence of the participation of ribosomes in the first step of protein glycosylation is presented.

The heterogeneous elongation factor eEF-1, isolated from Guerin epithelioma, can be separated into three fractions: one of them functionally corresponds to EF-1α, the second one to EF-1βγ, and the third is an unidentified, active aggregate named EF-1B, which contains the subunit forms EF-1α and EF-1βγ, and other polypeptides showing protein kinase activity. The aggregate EF-1B can be autophosphorylated, while the subunit forms EF-1α and EF-1βγ can neither become autophosphorylated nor phosphorylate other polypeptides. The subunit form EF-1βγ consists from two polypeptides of 32 and 51 kDa, corresponding to other eukaryotic β and γ polypeptides, respectively. EF-1βγ is thermostable and protects against thermal inactivation of EF-1α in the EF-1α–EF-1βγ complex.

Pure eEF-2 preparations isolated from normal and neoplastic tissues show different structural features. The existence of eEF-2 in multiple forms, differing in molecular mass, have been found. The eEF-2 with molecular weight of about 100 kDa can be phosphorylated, while eEF-2 of about 65 kDa was not phosphorylated by protein kinase eEF-2. The phosphorylated eEF-2 lost its activity, and this effect was reversed by dephosphorylation. The eEF-2 (65 kDa) was isolated from the active polyribosomes, and it may directly participate in the translocation step of the peptide elongation.

It was noted that the components of elongation system can be inhibited, in separate steps, by the substances isolated from various sources of plant origin. Alkaloids emetine and cepheline, cardiac remedy digoxin, saponin glycoside, and its aglycon directly inactivated ribosomes. Quercetin inhibited eEF-1 activity by directly influencing its subunit form EF-1α. eEF-2 was shown to be a target site of the inhibitory action of the glycoside isolated from *Melissa officinalis* leaves. [P.S.E.B.M. 1996, Vol 212]

Eukaryotic protein synthesis was widely and precisely described by Moldave (1). Translation of the codons in bound with ribosomes mRNA requires the presence of protein factors, GTP, a sulfhydryl compound, and various cations. Polypeptide elongation is the step of translation process. The

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two protein factors from rat liver required for polypeptide chain elongation have been designated (2, 3) as aminoacyl transferase I (elongation factor 1 [eEF-1]) and aminoacyl transferase II (elongation factor 2 [eEF-2]); they are analogous to factors designated T and G, respectively, from *Escherichia coli* (4, 5) and have been recognized in many other cells and organisms (6–8). Studies with highly purified translocation factors and with cell-free systems that translate a variety of mRNAs may provide insight into the regulatory mechanisms that affect protein synthesis at the level of these factors.

Translation occurs on ribosomes, small organelles that participate in a series of reactions that are repeated as each codon is translated sequentially, extending the nascent polypeptide chain from the N-terminal to the C-terminal residue.

In each iteration of the elongation, a peptidyl-tRNA is bound at first to a site on the ribosome called the P site. Next, an incoming aminoacyl-tRNA bearing the amino acid specified by the next unread codon on the mRNA binds to the A site, where its amino acid accepts the nascent chain. In bacterial systems, the existence (probably on the external surface of the small subunit) of the tRNA recognition site (R or pre-A binding site) is proposed (9), to which aminoacyl-tRNA is bound and then transferred to the A site.

In the elongation process, eEF-1 is responsible for the aminoacyl-tRNA binding to the ribosomes, while eEF-2 catalyzes the translocation of peptidyl-tRNA from the A to the P site. Peptidyl transferase, an enzyme integrally bound with ribosome, participates in the transfer of peptidyl-tRNA from the P to the A site and catalyzes peptide bond formation. It has been suggested (10) that the 23S ribosomal RNA participates in the peptidyl transferase function.

Ribosomes

Structural and Functional Elements. The structure of ribosomes evidently affects their function. A detailed description of the ribosomes was given by Lake (11). Ribosomes are organelles found in all cells and are the site of protein biosynthesis. They are the main subcellular particles where peptide initiation, elongation, and termination occur. The eukaryotic ribosomes are composed of the small and large subunits. In the eukaryotic small subunit are a single rRNA molecule (18S rRNA) and 30 proteins. The large subunit consists of three rRNAs (28S, 5.8S and 5S) and 40 proteins. The most distantly related organisms have similar, 3-dimensional ribosomal structure. During protein biosynthesis, the primary function of the small subunit is to hold the mRNA and the tRNAs, while the large subunit catalyzes the formation of peptide bonds. So far, at least two sites on the ribosome have been definitively shown to play a role in the elongation process: the A site, or the acceptor site, is responsible for the binding of the incoming aminoacyl-tRNA, and the P site, the donor site or peptide bearing site, is where the peptide joins with the aminoacyl-tRNA bound to the A site during the peptide elongation process. Additional sites, and their possible roles, have been postulated but not completely confirmed: the R site, or the recognition, entry, or pre A-binding site, to which aminoacyl-tRNA is bound and then transferred to the A site (9), and the E site, or the exit site, which is located next to the P site (12, 13). Deacylated tRNA from the P site is proposed to shift to the E site prior to its removal into the medium. The signal to remove deacylated tRNA from the E site may be the binding of the incoming aminoacyl-tRNA molecule to the A site. So far, data regarding multiple sites has only been obtained from prokaryotic systems, but such a model would clearly have implications for the mechanism of action of eukaryotic ribosomes as well.

The nascent polypeptides may fold on a ribosome, and this folding may take place in a "folding domain" of the large ribosomal subunit. According to the vectorial folding hypothesis, the folding of nascent peptides into specific domains of the native protein may occur during the nascent peptide formation on ribosomes (14).

It was shown that three tRNA molecules could be bound simultaneously to the same ribosome. The center portion of the amino acid of aa-tRNA, which had been bound to a ribosome as a peptidyl-tRNA analog, moved relative to the ribosome as the peptidyl transferase reaction took place. This displacement model further suggests the existence of three sites on ribosome (15). According to this model, the nascent peptide stays in one position when the chemical reaction of peptide transfer to an incoming aminoacyl-tRNA takes place, and the necessary movement is attained by change of tRNA position (16).

The results presented by Odom *et al.* (17) have shown that one of each molecule, deacylated tRNA, N-acetylPhe-tRNA (an analog of peptidyl-tRNA), and puromycin (an analog of aminoacyl-tRNA), could be bound simultaneously to the same ribosome. Noller *et al.* (18) have proposed the hybrid state model with three tRNA-binding sites on large ribosomal subunit and two on the small subunit.

By immunoelectron microscopy the mapping of the specific ribosomal proteins and their localization in the 3-dimensional ribosomal structure was possible. In the ribosomes of all organisms exist two functional (translational and exit or secretory) domains, located on the opposite ends of the ribosome (11). Detailed information is available on the translational domain, but rather little is known about the exit domain. Many

of the newly discovered morphological features of prokaryotic ribosomes could be similar to those of eukaryotic ribosomes. The structural features present in the translational mechanisms might function similarly in both types. This similarity may be possible because the translational domain, are similar in both, even if the initiation step is quite different. In contrast to this, the ribosomal organization involved with protein secretion could differ greatly in prokaryotic and eukaryotic ribosomes, because the rough endoplasmic reticulum does not exist in the prokaryotic cell (19).

Immunochemical methods were applied in the comparative studies on ribosomes isolated from various organisms. It was noted that antibodies specific for the ribosomes isolated from chicken and rat livers recognized only 20% of each other's determinants (20). Rat liver polysomes, containing nascent peptides of phenylalanine hydroxylase, were precipitated by purified immunoglobulin directed against the protein of this enzyme (21); then the specific mRNA, released from the immunoprecipitate, was used for synthesis and cloning (22, 23). By the immunoprecipitation of polysomes from embryonic chick skeletal muscle, mRNA was purified and then applied as a template for the synthesis of the heavy polypeptide chains of myosine (24).

Although the role of ribosomes is well known, their participation in protein glycosylation is rather unknown. It has been shown that ribosomes are labeled *in vivo* with [^{14}C]glucosamine; it is possible that the [^{14}C]glucosamine incorporation may be a result of the binding of the first *N*-acetylglucosamine to the nascent peptide, which is still present at the ribosomal site (25, 26). Preliminary results have shown that even highly purified ribosomes, free of glycosyltransferases, can bind UDP-*N*-acetylglucosamine and UDP-*N*-acetylgalactosamine. The formation of the ribosome-sugar binding did not require the participation of glucosyltransferases, which are integrally bound to the reticulum endoplasmic membranes and are required for further steps of protein glycosylation. The results have shown (27) that UDP-*N*-acetyl-[^{14}C]sugar is bound to the ribosomes and that this is neither unspecific adsorption nor incorporation of the labeled sugar into nascent polypeptide by glycosyltransferase. It may be assumed that such a ribosome, charged with a sugar nucleotide, may play the role of carrier or sugar donor for the lipid intermediates and/or for nascent polypeptide chains. So, the ribosome–UDP-*N*-acetylglucosamine complex may be instrumental in the glycosylation of the nascent peptide synthesized on the ribosome.

The portion of the sugar molecules that is bound with ribosomes can associate with exogenous lipid acceptor (dolichol phosphate [Dol-P]) and may be utilized for nascent peptide glycosylation (28). The labeled sugar, in the presence of glycosyltransferases, can be transferred from Dol-*P*-*P*-*N*-acetylglucosamine to the nascent polypeptide synthesized on the ribosome (28). These results allow us to conclude that (i) the ribosome bound to UDP-*N*-acetylglucosamine is utilized in the dolichol route for *N*-glycoprotein synthesis and (ii) the ribosome–UDP-*N*-acetylglucosamine complex may play a role in the first step of protein glycosylation.

Ribosomal Proteins. Ribosomal proteins play a fundamental role in the formation and stabilization of ribosome structure, and they take part in the regulatory processes (29). Several proteins, named ribophorins, specifically interact with the receptors of endoplasmic reticulum (30). The studies on the structure and function of ribosomal proteins have been considerably developed, but the problem of heterogeneity of protein composition in eukaryotic ribosomes is still unresolved to the end (31).

Differences between the ribosomal protein composition of neoplastic and normal tissue have been noted. Significant differences have been found in the group of acidic ribosomal proteins isolated from Guerin epithelioma cells (32). Detailed investigation, *in vivo* and *in vitro* (33–36), have shown that the acidic ribosomal proteins from tumor cells were phosphorylated more extensively than those of normal cells. In the Guerin epithelioma ribosomes, at least three additional protein fractions were observed, which when analyzed immunochemically (37, 38) seemed to be specific for the ribosomes of this tumor (32).

Diagnostic Meaning of the Ribosome Investigations. Antibodies against ribosomes were shown in patients with virus-induced autoimmune hepatitis (39), lupus erythematosus, and other collagenoses (40–43). Monoclonal antibodies against ribosomal proteins have been obtained (44) and used in diagnostics. Studies on the binding of antibodies to ribosomal proteins have been carried out in various diseases (45), and they form the basis for a preliminary opinion on the level of antiribosomal antibodies that can exist in the sera of a given portion of the human population.

The immunochemical properties of ribosomes can be utilized in therapy. Despite a large number of stipulations, preliminary experiments have suggested suitable application for a vaccine containing ribosomal antigens in some diseases. Good results were obtained in the therapy of bronchial asthma with the use of bacterial ribosomal antigens (46). It can be supposed, that such kind of vaccines can be very useful in the therapy applied in various fields of medicine.

Elongation Factors

Elongation factors (prokaryotic and eukaryotic) are members the class of proteins known as G-proteins. They utilize GTP in the binding of aminoacyl-

tRNA to ribosomes and in the translocation of peptidyl-tRNA from the A to the P site on the ribosome (47). Like other G-proteins, eEF-1 and eEF-2 hydrolyze GTP. However, they only hydrolyze GTP when they are in contact with ribosomes.

G-proteins form a class of proteins that are present in a range of systems, such as signal transduction, protein synthesis, and the cytoskeleton. G-proteins may form an important component of integrated systems in cells involved in the regulation of many biochemical events.

The common features of all G-proteins may be: binding of GTP and then association with other proteins; hydrolysis of GTP and dissociation from other proteins; and control through the protein-protein interactions. Thus, G-proteins can be regulatory, transducing proteins. The energy released after hydrolysis of GTP, bound with G-protein, seems to be necessary only for turning-off the system or recycling GTP. γ-Phosphate group is released as inorganic phosphate ion or, in some events, is used to phosphorylate another molecule.

G-proteins (subunits α, β, and γ) are heterotrimeric guanine nucleotide–binding proteins that function as molecular switches in a diverse set of signaling pathways by coupling heptahelical receptors to specific intracellular effectors. The transduction of signals depends on the ability of the α subunits to cycle between a resting (GDP-bound) conformation and an active (GTP-bound) conformation (48, 49). In response to extracellular signals, heptahelical receptors catalyze the exchange of GTP for GDP, resulting in an active conformation and greatly reduced affinity for βγ subunits (50). Crystal structures of GDP- and GTP-bound forms have been reported (51–54).

Heterotrimeric G-proteins are members of a large superfamily of GTPases which mediate processes such as cellular signaling and protein synthesis (55). Each protein in the GTPase family is a molecular switch that can change its affinities for other macromolecules. Turned on by binding GTP and off by hydrolyzing GTP to GDP, this switch mechanism is remarkably versatile. Specific member of the GTPase superfamily are able to alter between "ON" and "OFF" states (i.e., the active GTP conformation and inactive GDP conformation) (55).

Among the superfamilies are the small guanine nucleotide–binding proteins (molecular mass: 21,000) like the Ras proteins, including p21*ras*, which are products of the *ras* oncogenes. They have been found to be mutated in a large number of human tumors. The other classes of the GTPase family are α subunits of the heterotrimeric signal transducing G-proteins, and the translation factors (EF-Tu, EF-1α, EF-G, eEF-2, IF-2, and release factor RF-3) (56). Crystal structures of p21*ras* and their similarities with EF-Tu and α-chains, were described in detail by Bourne *et al.* (55). The EF-Tu is over twice the size of p21*ras*.

The 3-dimensional structure of EF-Tu is organized in three domains (57) and it will be characterized in the following section on elongation factor eEF-1. Domain 1 has a tertiary structure closely resembling that of p21*ras*. The topological order of secondary structure elements of p21*ras* is the same as that of domain 1 of EF-Tu (amino acids 1–166), but their primary structures are only 30% identical (55).

The guanine nucleotide–binding site in various G-proteins is rather similar, so the domain 1 of EF-Tu may be analogous to the binding site in eukaryotic EF-1α.

Functional cycle of the ON/OFF switch involves interactions of G-proteins with three components: (i) the effector to which the ON signal is transmitted, (ii) GTPase-activating protein (GAP), and (iii) with a guanine nucleotide exchange factor (GEF) to catalyze the exchange of GDP to GTP.

Elongation Factor eEF-1. *Structural Features.* The eEF-1 is heterogeneous, and the first information on its multiple forms in eukaryotic cells was applied by Schneir and Moldave (58). The heterogeneity of eukaryotic EF-1 was also described by Collins *et al.* (59) and others (60–65). Multiple molecular weight forms of eEF-1, referred to as EF-1H, have been isolated from rat liver (58, 66), pig liver (67–69), rabbit reticulocytes (70–71), silk gland (72), wheat embryo (73, 74) and calf brain (61). Various species of EF-1H have a common subunit of approximately 50 kDa, designated EF-1α (75), two other polypeptides of 33 kDa, identified as EF-1β, and a 48-kDa protein known as EF-1γ (76). Both EF-1H and EF-1α bind to GTP, but only the monomer forms a stable ternary complex with aminoacyl tRNA and GTP (75). Yeast EF-1βγ is catalytically equivalent in function to EF-1β and the function of EF-1γ is still not elucidated (76).

The EF-1α subunit is functionally identical to the EF-Tu from *E. coli*. EF-Tu is a monomeric protein of 43 kDa and is the most abundant protein in the cell, comprising as a much as 5% of total cell proteins. This factor is required as a key factor in maintaining translational fidelity (77) and it has been shown that two separate genes, tuf. A and tuf. B, code for this subunit form (78). It should be noted that EF-Tu, like other members of the GTPase superfamily, has intrinsic GTPase activity (79), which is greatly enhanced when this factor, complexed with aminoacyl-tRNA and GTP, binds to programmed ribosomes (80). Crystalographic investigations of intact EF-Tu have shown that its crystal structure consists of three separate domains (81, 82). Domain 1 is α and β structure alternated domain and is the locus of the GDP/GTP-binding site and of GTPase activity; it has a tertiary structure and is localized at the N-terminal end of the polypeptide. Do-

main 1 has a surface to surface contact with domain 3 (82). Domains 2 and 3 have antiparallel β-barrel structure. The association between domains 2 and 3 is relatively tight, but domain 2 is separated from the nucleotide-binding domain 1 (G-domain). Cross-linking studies implicated an interaction of domain 2 and 3 of eukaryotic EF-1α with aminoacyl-tRNA (83). All three domains are involved in tRNA binding, with the aminoacyl end interacting with domain 1, close to the C-terminal part of the effector region.

Heterogeneous eEF-1 from rat liver and from Guerin epithelioma has been separated into three or two subunit fractions. One of them functionally corresponds to EF-1α (84, 85); the second one stimulated EF-1α activity (85) and functionally corresponds to EF-1β(βγ) from other animal tissues. The third fraction, which was also active in the elongation process, is an unidentified aggregate, named EF-1B aggregate. eEF-1 from rabbit reticulocytes has also been dissociated into three subunit forms (86). Nombella *et al.* (62) have shown that eEF-1 in the tissues appears mainly in aggregates, the dimensions of which differs with the kind of the tissue.

The components of EF-1B aggregate were identified by Marcinkiewicz *et al.* (87). The EF-1α was obtained after dissociation of EF-B aggregate in the presence of GTP; its monomeric form may be also separated after chromatography of the heterogeneous preparations eEF-1 on a DEAE-Sephadex A-50 column (88). In higher eukaryotes, transfer RNAs, aminoacyl-tRNA synthetases, and elongation factors form a kind of "super structure" surrounding the ribosome as cloud, thereby promoting the efficiency of protein synthesis of the eukaryotic cell (89–91).

It was observed that eEF-1 can be changed in form during germination of wheat seeds and that during development EF-1α becomes dominant in cell extracts (92).

A model has been presented for the quaternary structure of eEF-1, including the interaction sites among the different subunits. The presence of two molecules of EF-1α in eEF-1 were found, and each of the two EF-1α molecules behave differently with respect to their substrates, aminoacyl-tRNA and GTP (93). According to this model, eEF-1 consists of a pentameric complex, composed of four different subunits EF-1α2βγδ. One molecule of EF-1α dissociates easily from this complex under the influence of aminoacyl-tRNA and GTP, while the second molecule of EF-1α was found to remain firmly attached. The way in which EF-1α dissociates from the eEF-1 complex under influence of charged tRNA and GTP is different from that in bacteria. Of the two EF-1α molecules bound to eEF-1 complex, only one can form a ternary complex with charged tRNA and GTP.

Functional Characteristics. Elongation factor eEF-1 is the enzyme responsible for the binding of aminoacyl-tRNA (other than the initiator Met-tRNA) to the acceptor A site on the ribosome. This process, in eukaryotic protein chain elongation, requires the intermediary formation of a ternary complex among the aminoacyl-tRNA, the GTP, and the binding factor eEF-1.

It seems that at least two forms of eEF-1 play a specific role in polypeptide chain biosynthesis. The EF-1α is the subunit form that participates in formation of a ternary complex (73, 94–97) *via* formation of a binary complex with GTP (95–100). EF-1α binds GTP, and the resulting binary complex reacts with aminoacyl-tRNA to form a ternary complex; this complex binds to and delivers the aminoacyl-tRNA to the ribosomal particle (65, 101–103), GTP is hydrolyzed (65, 96, 104), EF-1α–GDP complex is formed (97), and the factor is released from the ribosome (104). The other subunit EF-1β, catalyses the GDP/GTP exchange on EF-1α (64, 72, 105–107). According to this model, EF-1β reacts with EF-1α–GDP complex, displacing GDP and forming an EF-1α–EF-1β binary complex; then, GTP displaces EF-1β, resulting in the formation of an EF-1α–GTP complex which can bind another aminoacyl-tRNA.

The heteromeric complexes EF-1βγ and EF-1βγδ function in recycling the inactive EF-1α–GDP intermediate to the active GTP-bound form. Both subunits EF-1β and EF-1δ display the same degree of exchange activity, but they differ with respect to the mode and strength of their interaction with EF-1γ (108).

In tissue, where GTP/GDP ratio is high, an auxiliary factor like EF-1β may not be necessary for the regeneration of EF-1α–GTP complex. In yeast, most of the EF-1α is isolated in the monomeric form EF-1α, which is active in the absence of added EF-1β (109).

EF-1B aggregate from Guerin epithelioma cells (87) seems to be similar to EF-1H, which is known as a high-molecular weight complex containing EF-1α and EF-1β(βγ) subunit forms (110–112). The EF-1B aggregate was markedly more active in polyphenylalanine synthesis than EF-1α and has been shown to consist of at least three polypeptides separable by electrophoresis: one of them was EF-1α subunit, while the other two bands were the polypeptides of EF-1βγ. Another aggregate, named EF-1B′, which was also active in polyphenylalanine synthesis, was separated from the crude eEF-1 preparation (113). EF-1B and EF-B′ aggregates contain both subunit forms EF-1α and EF-1β(βγ), and other polypeptides. EF-1B and EF-1B′, but not EF-1α, can undergo autophosphorylation, because they contain protein kinase activities and can be phosphorylated without addition of the heterogeneous enzyme, as was confirmed on the autoradiographic study (113). In EF-1B′ two polypeptides

(one of approximately 32 kDa, corresponding to EF-1β, and another of approximately 90 kDa) were phosphorylated, while in EF-1B only the polypeptide of approximately 51 kDa, corresponding to EF-1γ, was phosphorylated. EF-1α and EF-1β, individually, were unable to become autophosphorylated and they cannot phosphorylate other polypeptides. This is in agreement with the suggestion of Janssen *et al.* (114) that the protein kinase phosphorylating EF-1β is a protein integrally bound to this factor, but does not have any function in the elongation cycle. Heparin, a specific inhibitor of the casein kinase II (CKII) (114, 115), distinctly inhibited autophosphorylation of EF-1B′ (113), so CKII should be present in this aggregate. The kinase, present in EF-1B, has not been classified. It was only shown that the histone H2A can serve as a substrate of the enzyme responsible for autophosphorylation of the aggregate EF-1B (113).

It was noted that polyphenylalanine synthesis was decreased when autophosphorylated instead of nonautophosphorylated EF-1B′ aggregate was used. This effect was not observed in the presence of autophosphorylated EF-1B (113). It was concluded that in the process of autophosphorylation of EF-1B′, its subunit form EF-1βγ was probably modified and inactivated. Phosphorylated subunit form [^{32}P]EF-1βγ was unable to form the EF-1α–EF-1βγ intermediary complex and the formation of EF-1α–GTP active complex was stopped. It seems that the autophosphorylation/dephos-phorylation of EF-1B′ aggregate may be a regulatory process in the polypeptide elongation step of protein biosynthesis.

In Guerin epithelioma, the high tendency of subunits EF-1α and EF-1βγ to form aggregates suggests some analogies with the early developmental period of lower eukaryotic organisms (115, 116), in which EF-1 exists as a high molecular complex EF-1H, which during the growth season is transformed to the lighter form. However, EF-1H has low activity and may represent some kind of storage form of EF-1L, which can be activated only when separated from other proteins. On the other hand, eEF-1 isolated from Guerin epithelioma was highly active in all aggregate forms.

Marcinkiewicz *et al.* (88) observed that EF-1βγ from tumor cells can be isolated from EF-1B aggregate (which consists of the subunit forms EF-1α and EF-1βγ) by dissociation of this aggregate in the presence of GTP (110). EF-1βγ from rat liver did not show this tendency to aggregate with EF-1α, and it can exist in a free form or aggregated with other proteins; it may be isolated without the GTP-dependent dissociation step (74, 106, 110, 117, 118). The EF-1βγ was resolved into two polypeptides (88) according to the method of reversible denaturation in 6 *M* urea (117, 119). The 32-kDa particle (polypeptide β) stimulated the GDP/GTP exchange, while the 51-kDa polypeptide (γ) was inactive in this process. In conformation previous work by Janssen and Moller (119), γ-peptide was shown to be hydrophobic. It was also noted that EF-1B aggregate is thermostable (88), which could suggest that thermostable EF-1βγ protects EF-1α against thermal inactivation. Slobin *et al.* (120) have shown that the aggregate of EF-1 from *Artemia salina* was not inactivated at 44°C, but upon addition of GTP the aggregate dissociated and lost its activity. Further studies may show whether polypeptide γ, the function of which is still unknown, is responsible for the thermostability of EF-1B aggregate.

There is evidence that EF-1 may also play a role in transcription (121). About 20% of the total cellular amount of EF-1α was found to be associated with the nuclear fraction. The results indicate that EF-1α may function in both nuclear transport and transcription; further research is needed to elucidate the whole of EF-1α's role in RNA synthesis (93).

Elongation Factor eEF-2. *Structural Characteristics.* Preparations of eEF-2, isolated from various sources and purified to a homogeneous state by different methods, differ in molecular masses: 100 kDa from rat liver (122) and rabbit reticulocytes (123), 80–96 kDa from hen oviduct (124), 85 kDa from calf brain (110), and 65 kDa from rat liver (125).

The eEF-2 isolated from hamster is a single polypeptide consisting of 857 amino acids with a highly conserved sequence (126). Yeast and human eEF-2 are 85% homologous (127).

The GTP-binding site and GTPase activity are localized in the conserved region G1–G5, containing 160 aminoacyl residues on the N-terminal end of the eEF-2 polypeptide chain (126, 127). Region G1 is probably responsible for GTPase activity, while G2–G3 contains the guanine binding site. The region G1–G5 is homologous with other GTP-binding proteins: EF-TU, EF-G, EF-1α, IF-2α, and Ras (57).

Region E, which is localized in the N-terminal fragment of the eEF-2 molecule and half of the C-terminal part of the molecule, homologous with prokaryotic EF-G, is responsible for the binding of eEF-2 to ribosomes. This region includes 54–78 positions of aminoacyl residues and is homologous with the sequence found in elongation factors EF-1α, EF-Tu, and EF-G. Arginine, localized in the number 66 position, takes part in the interaction of eEF-2 with ribosomes and is the first site of the digestion of this factor by trypsin (128). The alkylation of the cysteine residue prevents eEF-2–GTP formation with ribosomes, which indicates that these residues have a role in the binding of eEF-2 with ribosomes (129). The domain of eEF-2, with histidyl residue in the number 715 position (130) and with a threonyl residues in the region of positions 51–60 (131), is responsible for the biological regulation of this factor. The EF-G protein is highly

elongated and composed of five domains. Two domains are related to ribosomal proteins and RNA-binding proteins; two are similar in structure to EF-Tu (54). The GDP-binding fragment of EF-G, like that of other members of the GTPase family (132), is the N-terminal, nucleotide-binding domain or G-domain. In domain 5, as in domain 3, there is a high level of sequence similarity between EF-G and eEF-2, comparable to the level in core of the G-domain (132).

The eEF-2 can be markedly altered in some organisms, for instance in mutants (133) or under changed conditions (134–139). Tumoral eEF-2, purified from Guerin epithelioma cells has been characterized as homogeneous protein consisting of identical polypeptides with molecular mass, isoelectric point value and amino acid composition very close that of rat liver (125, 140, 141).

N- and C-terminal fragments of the eEF-2 molecule are important in its functional activity and in its interaction with ribosomes (142). In eEF-2 preparations, isolated from various sources, was only one N-terminal amino acid (138, 139, 141, 142). In both preparations of eEF-2, from Guerin epithelioma and rat liver, the only N-terminal amino acid was alanine (141). The C-terminal amino acid in eEF-2 from rat liver was glycine, while in eEF-2 from Guerin tumor cells it was serine (141). The eEF-2 from tumor cells was less sensitive to degradation by carboxypeptidase than that of rat liver cells (143). Evidence that the portion of the eEF-2 structure responsible for the binding reaction with ribosomes may be situated at the C-terminal end of the molecule (126) may lead to an understanding of the structural differences of eEF-2 preparations isolated from normal and Guerin epithelioma cells.

Gajko *et al.* (144) have shown the possibility the eEF-2 exists in multiple forms. During copurification, two active polypeptides were separated, electrophoretically and by ultracentrifugation, into bands of approximately 100 and 65 kDa (144). Thus, eEF-2 exists in at least two forms differing in molecular mass. Hradec (136) has also shown the existence of two different translocases in mammalian tissues. One of them corresponds to that of an earlier purified translocase (125), and the other ($S_{20,\omega}$ = 2.6S) was interpreted as an active subunit of the other translocase (136). The functional relations between translocation factor (2.6S) and factors eEF-2 (100 and 65 kDa) have not been investigated. First, the question was whether both eEF-2 forms (100 and 65 kDa) are bound to the ribosome and which of them takes part directly in the translocation process. It was shown that the endogeneous ribosome-bound eEF-2, isolated from the active polysomic fraction, was only the active protein having molecular weight of about 65 kDa (144). It seems that from these two multiple forms existing in the rat liver cells only the eEF-2 of about 65 kDa is bound with ribosomes as an active form and it may take part directly in the translocation process. Furthermore, it was found that the only eEF-2 (100 kDa) was phosphorylated by kinase of eEF-2, while eEF-2 (65 kDa) was not phosphorylated at all (144). This finding can be important for further studies concerning the regulatory role of the phosphorylation/dephosphorylation process of eEF-2.

Functional Features. Elongation factor eEF-2 participates in the elongation of ribosome-bound polypeptides, together with eEF-1 and GTP; it is involved in translocation of mRNA and peptidyl-tRNA from the aminoacyl A site to peptidyl P site movement of message and removal of free tRNA from the P site, which allow the ribosome to bind new incoming aminoacyl-tRNA at the aminoacyl site (145–148).

The eEF-2 makes a complex with GTP that very quickly binds to the ribosome (149). The ternary complex, eEF-2–GTP–ribosome, induces the GTPase activity and is transformed during the translocation step into the eEF-2–GDP–ribosome complex, which becomes dissociated into ribosome and the binary complex eEF-2–GDP. Later, GTP reproduces the eEF-2–GTP complex, by displacing GDP. Evidence has been presented (150) that during the translocation process, a single enzyme (eEF-2) catalyzes the translocation of several ribosomes, suggesting that the interaction between ribosomes and eEF-2 is a reversible process, and that the active eEF-2 dissociates from the complex on formation of the product. The eEF-2–ribosome complex would dissociate when the substrate of the reaction, peptidyl-tRNAa, becomes converted to peptidyl-tRNAp; the released eEF-2 could then react with the same or another ribosome.

The elongation factor eEF-2, as the member of G-proteins, belongs to the class of proteins with GTPase activity (56). It is able to change its own conformation from inactive with GDP to active with GTP. For eEF-2–GDP, in contradistinction to eEF-1α–GDP, a separate guanine nucleotide exchange factor for catalyzing the exchange of GDP to GTP was not discovered. eEF-2, similar EF-G in prokaryotic system, is exceptional among GTPases since it does not have a GEF (GDP/GTP exchange factor), whereas EF-Tu requires elongation factor EF-Ts as a specific GEF. It has been claimed that the relatively lower affinity of EF-G for GDP abolishes its need for GEF (55).

Studies on specificity of the elongation factors have shown that the prokaryotic EF-T can replace EF-1 in animal systems, while EF-G from *E. coli* was inactive when added instead of animal EF-2 (133). Cross-examination between elongation systems isolated from rat liver and Guerin epithelioma cells has shown a lack of tissue specificity. However, affinity of

the Guerin eEF-2 to the liver elongation system was lower by half compared with the homologous tumor or regular liver systems (141).

It has also been shown that eEF-2 is the specific target of diphtheria toxin, which inactivates the factor by ADP-ribosylation (135). Ultracentrifugation studies with the use of pure eEF-2 isolated from the rat liver (125) suggest that the formation of a ternary complex consisting of the toxin, eEF-2, and NAD^+ may be the first step of this inactivation (134). This complex dissociates when the nicotinamide riboside linkage is cleaved resulting in formation of the ADP-ribosylated eEF-2. This may indicate that the mechanism of inactivation may not involve simply a transfer of ADP-ribose from the toxin to eEF-2.

The ADP-ribosylated eEF-2 is impaired in ribosomal binding (151) and the target residue has been posited to be essential for the translocase activity of the factor (130). A reversible ADP-ribosylation of the same residue by cellular mechanism may be important for regulating the activity of eEF-2 in eukaryotic cells (152).

Since GTP-hydrolysis follows the translocation step, it is possible that protein synthesizing systems can be poisoned by fusidic acid (153). Fusidic acid could inhibit synthesis by preventing the conformational change that normally follows GTP hydrolysis. Fusidic acid binds to the EF-G–GDP–ribosome complex and blocks elongation by preventing dissociation of the complex.

Many proteins of the eukaryotic translational system, including eEF-2, can be phosphorylated. It appears that eEF-2 phosphorylation may be involved in the regulation of protein biosynthesis and other processes.

In various animal tissues, the existence of an identical substrate of 100 kDa, a specific substrate for the calmodulin and Ca^{2+}-dependent protein kinase has been shown, and this substrate was identified as eEF-2 (154). It also seems to be the first indication that calmodulin can be involved in the regulation of translation (155). The special Ca^{2+}/calmodulin-dependent protein kinase that specifically phosphorylated eEF-2 was named protein kinase III (131, 156–158). Other protein kinases capable of phosphorylating eEF-2 may exist, but this phosphorylation is insignificant compared with phosphorylation by the kinase of eEF-2 (kinase III). Phosphorylation results in eEF-2 inactivation and the inhibition of protein biosynthesis (157, 159–162).

The phosphorylation of eEF-2 takes place only at sufficient concentrations of Ca^{2+}, and its level in the cell may be important in the regulation of the rate of protein biosynthesis. Increases of Ca^{2+} concentration in the cytoplasm of rabbit reticulocytes resulted in inhibition of peptide elongation (163). The elevation of Ca^{2+} in the cytoplasm is transient, so the phosphorylation and inhibition of protein biosynthesis is also transient. Stimulation of fibroblasts with bradikinin causes a transient rise in intracellular Ca^{2+} and results in the transient phosphorylation of eEF-2 (164). It is possible that the initial response of cells to hormones and growth factors, which induce a transient increase of cytoplasmic Ca^{2+} concentration, may be an inhibition of protein biosynthesis after phosphorylation of eEF-2 by Ca^{2+}/calmodulin-dependent protein kinase III. A transient stop of translation by Ca^2-dependent phosphorylation of eEF-2 allows the expression of new genes. The translation is blocked at the level of peptide elongation, but not at the level of transcription or initiation of translation; it is possible to stop protein synthesis immediately (165).

It was reported that the phosphorylation of a single threonine, Thr-56, is located in the so-called effector domain (126) and threonine residues (Thr-53, Thr-56, and Thr-58) are phosphorylated in the N-terminal part of the eEF-2 molecule (166). The phosphorylated eEF-2 is unable to catalyze the translocation process (167). The phosphorylated eEF-2 has a decreased ability to form a ternary complex with ribosomes, and further results suggest that, even in the absence of ribosomes, phosphorylated eEF-2 has a decreased affinity for GTP, but not for GDP (168).

The protein kinase of eEF-2 has been purified and partly characterized by Gajko *et al.* (169). In the initial step of the purification, the enzyme existed in an inactive form. Then, it was reactivated when separated from the inhibitory substance (169). The existence of the kinase of eEF-2 together with its inhibitor makes clear why this enzyme was inactive in the crude preparation. The purified kinase of eEF-2 was an electrophoretically homogeneous protein, similar to that described by Nilsson and Nygard (170, 171).

It should be pointed out that eEF-2 phosphorylation, as well as the Ca^{2+} level, can be regulated by changes in pH (172), dephosphorylation (162), concentrations of cAMP, inducing dephosphorylation of eEF-2 (159), and through phosphorylation of the kinase of eEF-2 itself (171). Kinase of eEF-2 must be phosphorylated to be active. This enzyme, when dephosphorylated by alkaline phosphatase, is completely inactivated, while after its incubation with cAMP-dependent protein kinase, kinase of eEF-2 activity is restored.

The existence of the phosphatase of eEF-2, which exhibits specificity toward eEF-2 and dephosphorylates this factor more rapidly than any other protein, was also described (173).

Additional Elongation Factor. An additional elongation factor, EF-3, was found in yeast cells (174). This factor is uniquely required by yeast ribosomes. Ribosomes from other eukaryotes do not require this

protein (175, 176). EF-3 stimulates binding of the ternary complex (EF-1α–aa-tRNA–GTP) to ribosomes (177) and it is a single polypeptide of molecular weight 125 kDa, and with isoelectric point 5.9. EF-3 is probably a loosely bound ribosomal protein required in the elongation process in yeasts (178). Inactivation of EF-3 by polyclonal or monoclonal antibodies immediately blocks the elongation process (179). Western blot analysis has shown, that anti EF-3–cross-reacting material is present only in yeast. The gene YEF-3, encoding EF-3, has been isolated from yeast (180) and shown to be a single copy gene. The precise function of EF-3 is not known at present time.

Substances of Plant Origin That Inhibit Peptide Elongation Process

Protein biosynthesis is the fundamental process in the cells of all organisms. This process can be regulated, stimulated, or inhibited by various substances. Plants were used as antitumor remedies in folk medicine for a very long time, but little research has been performed on their effects on protein biosynthesis, which proceeds very fast in tumor development. It has been shown that peptide elongation may be inhibited, in separate steps, by substances isolated from various sources of plant origin. Some of them directly inactivate ribosomes, others eEF-1 or eEF-2.

Flowers of *Verbascum thapsiforme*, actually used as an expectorant, were also applied in folk medicine as antitumor remedies. Paszkiewicz *et al.* have shown (181) that aqueous extract from these flowers inhibits protein biosynthesis and that the saponin fraction of this extract is responsible for the inhibitory effect. It was also shown that ribosomes directly treated with this fraction lost their activity markedly, probably as a consequence of the formation of the inactive complex with the inhibitory substance. Sapogenins, those isolated from the hydrolysate of saponin fraction as well as commercial saponin preparations, directly inactivated ribosomes, too. The greatest effect was when the aglycon of the saponin glycoside, 18-β-glycyrrhetinic acid, was used. This aglycon was more potent than the glycoside and may be mainly responsible for the inhibitory effect of the saponin. The binding of the ternary complex eEF-1α–GTP–[^{14}C]Leu-tRNA and eEF-2 to ribosomes was blocked by saponin substances that directly inactivated ribosomes (181). The indirect inhibition of the eEF-1 and eEF-2 probably resulted from the blocking of the binding site for these factors on the ribosome.

Alkaloids are the largest group of therapeutic substances of plant origin, but their side effects should be further studied. Chlabicz *et al.* (182) have shown that the solution of dry Ipecacuanhae extract inhibits protein biosynthesis *in vitro*. Ipecacuanhae alkaloids, emetine and cepheline, directly inactivated the ribosomes and are thus responsible for the inhibitory effect observed. According to earlier studies (183), Ipecacuanhae alkaloids can block the transfer reaction of aa-tRNA in protein biosynthesis. This is probably the result of blocking a site on the ribosome (182), where the quaternary complex of ribosome–eEF-1–GTP–aa-tRNA is formed during the peptide elongation process.

An inactive complex can be also formed in the direct reaction of Digoxin (12-β-hydroxydigitonin, a well known cardiac remedy) with ribosomes, in which the incorporation of [^{14}C]leucine into proteins is blocked (184).

A selective inhibitor of eEF-1 activity has not yet been discovered. Recently, it was demonstrated (185) that the Quercetin (3,3′,4′,5,7-pentahydroxyflavone) forms an inactive complex with EF-1α and inhibits [^{14}C]phenylalanyl-tRNA from binding with ribosomes *in vitro*. As a consequence of blocking the ternary complex EF-1α–GTP–Phe-tRNA formation, the quaternary complex EF-1α–GTP–Phe-tRNA–ribosome cannot be created. Some antibiotics (e.g., tetracyclines) can block the binding A site on the ribosome; however, eEF-1 still remained active (186, 187). Quercetin does not directly affect the ribosome activity, so its inhibitory target size is probably EF-1α.

The aqueous extract of *Melissa officinalis* can stop virus development and tumor cell division, and inhibits protein biosynthesis *in vitro* (188). This information inspired further investigations of the inhibitory substances contained in this plant. Chlabicz *et al.* (189) have shown that the glycoside fraction, isolated from *Melissa officinalis* leaves, is mainly responsible for the inhibition of protein biosynthesis. This glycoside diminished the amount of peptidyl-tRNA in the ribosomal P site with no influence on peptidyl transferase itself; it specifically effected eEF-2 activity, such that the translocation step and peptide elongation were stopped. This finding, along with the structural differences between tumoral and rat liver eEF-2 previously described (140, 141) could be used to search for specific inhibitors of protein biosynthesis in tumoral cells.

The eEF-2 molecule was also found to be an inhibitory target site for aloe—a very interesting raw material from plants, which has been employed a long time in various therapeutic situations including the treatment of tumor diseases. Aloin and aloeemodin, isolated from the dry aloe extract, are responsible for the inhibitory effect of this plant material (184).

To summarize, it is clear that several features of the elongation system, including the properties of ribosomes, elongation factors, and their interactions, as studied in both normal and tumor cells, can, along with a knowledge of how various substances isolated from plants can effect protein biosynthesis, provide a basis

for the study of specific inhibitors of protein biosynthesis in tumor cells.

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