Moonlighting Functions of Polypeptide Elongation Factor 1: From Actin Bundling to Zinc Finger Protein R1-Associated Nuclear Localization

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Eukaryotic polypeptide elongation factor EF-1 is not only a major translational factor, but also one of the most important multifunctional (moonlighting) proteins.

EF-1 consists of four different subunits collectively termed EF-1αβγδ and EF-1αβγδ in plants and animals, respectively. EF-1α-GTP catalyzes the binding of aminoacyl-tRNA to the A-site of the ribosome. EF-1βγ (EF-1β and EF-1β'), catalyzes GDP/GTP exchange on EF-1α-GDP to regenerate EF-1α-GTP. EF-1γ has recently been shown to have glutathione S-transferase activity.

EF-2 catalyzes the translocation of peptidyl-tRNA from the A-site to the P-site on the ribosome. Recently, molecular mimicry among tRNA, elongation factors, releasing factor (RF), and ribosome recycling factor (RRF) has been demonstrated and greatly improved our understanding of the mechanism of translation.

Moreover, eukaryotic elongation factors have been shown to be concerned or likely to be concerned in various important cellular processes or serious diseases, including translational control, signal transduction, cytoskeletal organization, apoptosis, adult atopic dermatitis, oncogenic transformation, inflammation, and nuclear processes such as RNA synthesis and mitosis.

This article aims to overview the recent advances in protein biosynthesis, concentrating on the moonlighting functions of EF-1.

Key words: protein biosynthesis; translation; elongation factor 1; cancer; apoptosis

1. Discovery and characterization of elongation factors

The existence of a soluble factor involved in the transfer of aminoacyl-tRNA (aa-tRNA) to the ribosome was suggested more than 40 years ago for rat liver cell-free extract. In 1964, the factor was resolved into two complementary factors, aminoacyl transferase I and II, which correspond to the present EF-1 and EF-2 (or eEF1 and eEF2 in a new terminology, IUBMB, 1995: Table 1), respectively.

Meanwhile, extensive purification and characterization of E. coli factors EF-T and EF-G was done. One of the most important accomplishments in the E. coli translation system was the resolution of EF-T into EF-Tu and EF-Ts, the former of which catalyzes the binding of aa-tRNA to the ribosome, and the latter catalyzes the exchange of GDP/GTP on EF-Tu-GDP.

EF-2 has been purified from various organisms and shown to be a nearly 100-kDa monomeric protein having translocase and ribosome-dependent GTPase activities.

EF-1 purified from rabbit reticulocytes was reported to be a trimer of identical 62-kDa subunits that efficiently catalyzed aa-tRNA binding to ribosomes, and EF-1 was shown to have nearly equal dissociation constants for GDP and GTP. Based on these results, the factor that corresponds to EF-Ts was thought to be absent in eukaryotes. However, we were struck by the idea that fundamental mechanisms such as polypeptide elongation would not be different in prokaryotes and eukaryotes, and consequently searched for a eukaryotic factor that corresponds to EF-Ts. However, the purification of eukaryotic EF-1 was greatly hampered by the facts that it has a heterogeneous molecular weight ranging from 50 to several hundred kDa, and that the subunit structure of EF-1 was unknown.

The key to solving problems was the observation that silk gland EF-1 consists of three different subunits (EF-1αβγ), and that EF-1γ, which corresponds to the present EF-1β', has GDP/GTP exchange activity, and stimulates EF-1α-dependent binding of aa-tRNA to the ribosome (Fig. 1). Finally, as summarized in Table 1, EF-1 was shown to consist of four different subunits, EF-1αβγδ in plants, and EF-1αβγδ (or EF-1αβγδ) in animals. Yeast EF-1 consists of three subunits, EF-1αβγ. The molecular masses of the rice EF-1αβγ subunits are 52, 29, 27, and 53 kDa, respectively. Although the terminology is somewhat confusing, EF-1δ and EF-1β from mammals correspond to EF-1β and EF-1β' from plants, respectively.

As EF-1βγ (EF-1β and EF-1β') catalyzes the GDP/GTP exchange reaction on EF-1α-GDP, which has been released from the ribosome after the EF-1α-dependent binding of aa-tRNA, EF-1βγ corresponds functionally to EF-Ts (Fig. 1). These results established the fact that there is a common polypeptide elongation mechanism in prokaryotes and eukaryotes. Although the function of the fourth subunit, EF-1γ (originally EF-1β), which has been detect-
ed only in eukaryotic EF-1, remains unknown, rice embryo EF-1γ has recently been shown to have a glutathione S-transferase activity (Section 3).

EF-3 has been detected only in fungi. It has a molecular mass of 116 kDa in yeast and has ribosome-dependent ATPase activity. EF-3 stimulates the binding of EF-1α-GTP·aa-tRNA to the ribosomal A-site by facilitating release of deacylated tRNA from the E-site on the ribosome.

In the last decade, EF-1α was shown to have remarkable functions in addition to its role in translation (Table 1). Multifunctional proteins like EF-1α have generated great interest and have been called moonlighting proteins.

2. Molecular Mimicry in Translation

The gathering together of the recently established "molecular mimicry" among prokaryotic translational factors and the precise mechanism of ribosome functions, the whole processes of polypeptide elongation comprising, "aa-tRNA binding", "GDP/GTP exchange", "peptidyl transfer", and "translocation" has been illustrated in Fig. 1.

From the strikingly high similarity of the tertiary structures among the EF-Tu·GTP·aa-tRNA ternary complex and EF-1α, EF-1γ, EF-1β, EF-1β subunits, EF-3, it has been recognized that EF-1α is a moonlighting protein and participates in the same "molecular mimicry" of EF-Tu and EF-1γ with EF-3 in translation and post-translational processes of a cell.

Table 1. Translational and Moonlighting Functions of Elongation Factors

<table>
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<td>EF-1γ (eEF1Bγ)</td>
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* New nomenclature of EF is shown in parentheses.
complex, EF-G, and releasing factor RF, marvelously "molecular mimicry in the translational system" has been discovered.\(^{20,21}\) In the mimicry (tRNA mimicry), protein and RNA (tRNA) emulate each other's structures and functions. EF-2\(\cdot\)GTP (EF-G\(\cdot\)GTP), which has structural similarity to peptidyl-tRNA, binds to the A-site and pushes off the peptidyl-tRNA from the A-site to the P-site concomitant with the movement of mRNA just one codon.

Surprisingly, RF recognizes termination codons using a "peptide anticodon" by a mechanism similar to that of codon recognition by tRNA.\(^{22}\) Recently, ribosome recycling factor (RRF), which in cooperation with EF-G catalyzes the recycling of the ribosome after one round of protein synthesis, has been also shown to mimic tRNA.\(^{23}\) RRF releases the tRNA remaining in the A-site after releasing a completed protein. The molecular mimicry among tRNA and translational factors is one of the most notable examples of "convergent evolution": the evolution of similar functional structures from different origins.

3. Structure and Functions of EF-1 and EF-1 Genes

As EF-1\(\alpha\) is ubiquitous and the second-most abundant eukaryotic protein after actin, comprising 1–2% of the total proteins in normal growing cells,\(^{24}\) its genes and the control of its expression have several characteristic features conserved among various organisms. As the eukaryotic counterpart of EF-Ts consists of three subunits, EF-1\(\beta\beta'\gamma\) (EF-1\(\beta\beta'\gamma\)), the evolution of each subunit and its functions are of great interest.

(1) Organization and structure of EF-1\(\alpha\) genes

EF-1\(\alpha\) is encoded by a small multigene family. There are two EF-Tu genes in E. coli, at least two EF-1\(\alpha\) genes in humans, four genes in rice, and more than 10 genes in maize. The deduced amino acid sequences of EF-1\(\alpha\) and EF-Tu from various species are highly similar; for example, the sequences in rice share 96, 78, 74, 51, and 30% identity with those of Arabidopsis thaliana, Homo sapiens, Saccharomyces cerevisiae, the archaean Pyrococcus woesei, and Escherichia coli, respectively.\(^{25}\) As elongation factor genes are ubiquitous and well conserved among organisms, they have been widely used for the phylogenetic analyses. There are two relatives, EF-1\(\alpha\) (EF-Tu) and EF-2 (EF-G) groups, enabling phylogenetic trees with a root to be drawn. The most prominent result obtained from such analyses has been the demonstration of the fact that archaean are located between eubacteria and eukaryotes.\(^{26}\)

EF-1\(\alpha\) genes may represent a model of the evolu-
tion of small multigene families. As the four rice EF-1α genes (refa1, 2, 3 and 4) comprise two sets of two tandemly arranged EF-1α genes, refa1-refa4, and refa2-refa3, it is likely that duplicated genes were further duplicated as sets to form four genes.\(^{25}\)

As EF-1α is the second-most abundant protein, the gene is likely provided with one of the strongest promoters. Furthermore, EF-1α mRNA has a specific sequence, called 5' TOP, which is responsible for efficient translation of the mRNA (Section 4).

In Xenopus oocytes, EF-1α S, which is expressed mainly in somatic cells, contains a G/C element and multiple G/C boxes in the enhancer and promoter, respectively. The G/C box binding protein Sp1 has been suggested to play a major role in the developmental regulation of the promoter. In contrast, the promoter of EF-1α O, which is exclusively expressed in oocytes and during early development, contains a CCAAT box, but does not contain either a TATA box or an Sp1-binding site.\(^{27}\)

A telo-box, i.e., the interstitial telomeric sequence AACCCTAA, has been found in the promoters of *Arabidopsis* and *Drosophila* EF-1α genes and of several highly expressed plant genes.\(^{28}\) Recently it has been suggested that the telo-box, in synergy with the cis-elements of the EF-1α, acidic ribosomal protein rp40, histone H4, and proliferating cell nuclear antigen (PCNA) genes, regulates the expression of the respective genes in *Arabidopsis* root meristems.\(^{29}\) As PCNA is a major gene product with the ability to bind DNA polymerase δ and to stimulate DNA replication, further elucidation of the function and mechanism of action of the telo-box in gene expression will contribute to our understanding of the coupling between DNA replication and translation in proliferation.

(2) **Structure and functions of EF-1α**

EF-1α is an ancestral GTP-binding protein (G-protein). EF-Tu and EF-1α are composed of three domains: I, II, and III. Domain I (G-domain) of G-proteins such as Ras and heteromeric G-proteins including EF-Tu and EF-1α contain consensus sequences, GxxxGKS (motif I or motif G-1), DxxG (motif II or motif G-3) and NKxD (motif III or motif G-4), which are responsible for the binding of GTP.\(^{30}\) Motif I is concerned with the phosphate binding and is called the P (PO\(_4^2-\)) box. Motif II, which is called the G (guanine) box, is concerned with the binding of the guanine ring. Motif II, which is called the S (switch) box, is concerned with GTP hydrolysis, and the α-helix (helix B) following the glycine in motif II undergoes a large conformational transition depending on whether GTP or GDP is bound to the EF-1α.\(^{31}\) The GTPase switch, which is found in all G-proteins, is one of the most widely used molecular devices in the control of cellular processes. In the inactive GTP-bound form, the domain I of EF-Tu is located apart from domains II and III. Upon binding GTP, the switch is turned on and domain I rotates approximately 90° toward domains II and III to form a cavity for the binding of aa-tRNA.\(^{32}\) The conformational changes of EF-Tu (EF-1α) during the execution of its functions, i.e., aa-tRNA binding to ribosome and the release of EF-Tu (EF-1α) itself from the ribosome, are among the most dramatic conformational changes found in proteins thus far.

(3) **Structure and functions of EF-1β and its homologues**

The amino acid similarity between the two similar EF-1β (EF-1β and EF-1β') is higher within a plant (rice) or animal (Artemia) than that between the same factors in the two kingdoms. These results indicate that the two similar factors of rice and *Artemia* have evolved independently after the two kingdoms were separated. As the similarity between rice and *Arabidopsis* EF-1β is higher than that between EF-1β and EF-1β' within each species, it is likely that plant EF-1β and EF-1β' evolved by duplication before monocots (rice) and dicots (*Arabidopsis*) were separated. Although rice EF-1β and EF-1β' are ubiquitously expressed, the third similar EF-1β, EF-1β2, which has higher similarity to EF-1β, is specifically expressed in rice seed and cultured cells.\(^{31}\)

Although homodimers of EF-Ts are detected,\(^{33}\) rice EF-1β and EF-1β' form heterodimers, but not homodimers, and EF-1β but not EF-1β', has the ability to form a complex with EF-1γ. By the sequential addition of EF-1γ and EF-1α to the EF-1βγ complex, EF-1αββ'γ, containing one mole of each subunit, has been reconstructed.\(^{34}\)

The mechanism of the GDP/GTP exchange reaction has been deduced from the crystal structure of the EF-Tu/EF-Ts complex. In this complex, Phe81 of EF-Ts is inserted into the hydrophobic pocket on EF-Tu, resulting in the release of the bound GDP from EF-Tu to form the EF-Tu·EF-Ts complex.\(^{35}\) In the presence of GTP and aa-tRNA, the complex changes to an EF-Tu·GTP·aa-tRNA ternary complex.

Recently, a similar GDP/GTP exchange mechanism has been demonstrated in eukaryotes by NMR analysis of the interaction between human EF-1α and the C-terminal half of EF-1β (134–224). Interestingly, striking similarity between the tertiary structures of EF-Ts and EF-1β has been demonstrated, despite the dissimilarities of their primary structures. In eukaryotes, Tyr181 of EF-1β, which is conserved among eukaryotes, has been thought to play an analogous role to Phe81 of *E. coli* EF-Ts.\(^{36}\) Using EF-1β' fragments expressed in *E. coli*, the EF-1β amino acid residues from 122 to 129 have been suggested to have GDP/GTP exchange activity.\(^{36}\) Thus, further extensive works will be necessary to analyze
of the entire GDP/GTP exchange mechanism in detail.

(4) Structure and functions of EF-1γ
EF-1γ is a monomeric protein of about 50 kDa. Although the physiological significance is unknown, there are many reports suggesting that EF-1γ may have some cellular regulatory functions: EF-1γ has the ability to bind to microtubules, and EF-1γ mRNA is over-expressed in carcinomas (Section 6), and the consensus amino acid residues that are necessary for the glutathione S-transferase (GST) activity are conserved in EF-1γ from various eukaryotes. The last result is the most interesting, as it suggests the existence of GST activity in EF-1γ.

GST catalyzes the conjugation of electrophilic xenobiotics or endogenous compounds with glutathione, and plays various physiological roles in the detoxication of potential alkylating agents, prostaglandin biosynthesis, and anthocyanin biosynthesis. Although the GST activity is detected in the EF-1γ-containing subcellular fraction adsorbed to a glutathione-agarose column, the presence of intrinsic GSTs in this fraction has not been ruled out. Attempts to express EF-1γ, which has GST activity, in E. coli have been unsuccessful. Recently, we obtained data showing the existence of GST activity in both EF-1βγ and EF-1γ expressed in E. coli. Although the function of the GST in EF-1γ is unknown at present, it may be a sensor that signals the redox state of the cell to the protein synthesizing machinery, as translation is activated by reduced glutathione and inhibited by oxidized glutathione.

(5) Higher organization of translational factors
Various aa-tRNA synthetases have been shown to be complexed with EF-1. As EF-1α can also form complexes with free tRNA, and EF-1α has the ability to stimulate aa-tRNA synthesis, these translational components are most likely organized in a highly ordered complex to facilitate efficient aminoacylation and subsequent transfer to EF-1α. Interestingly, even the tRNA in the exit site (E-site) on the ribosome has been suggested to be delivered directly to EF-1α without being released to the cytoplasm in a free form (Fig. 1). The tRNA-EF-1α complex is thought to be integrated into an aa-tRNA synthetase complex, and subsequently to be aminoacylated. The finding that aa-tRNA added externally to permeabilized Chinese hamster ovary (CHO) cells was not used for protein synthesis supports the idea that translational factors are organized within the cell, and free aa-tRNA may not be integrated into the presumptive complex.

4. Translational Control and Elongation Factors

As transcription and translation are spatio-temporally different in eukaryotes, the control at the translational level has greater importance in eukaryotes than in prokaryotes, in which these processes proceed nearly simultaneously. Not only does the modification of elongation factors participate in the control of the rate and accuracy of translation, but also the synthesis of elongation factors itself is translationally controlled. As the study of translational control is a big research field, this review will discuss only cases in which elongation factors are concerned directly.

(1) Phosphorylation of elongation factors
Each of the EF-1 subunits and EF-2 are phosphorylated and dephosphorylated by various protein kinases and phosphatases, respectively, depending on the cellular lineage, cell cycle, developmental stage, and environment. EF-1α is activated by phosphorylation with protein kinase Cδ (PKCδ) or S6 kinase. EF-1βγ is activated by phosphorylation of the β and γ subunits with S6 kinase (or casein kinase 2) and cdc 2 kinase, respectively. During meiosis in *Xenopus*, the EF-1γ subunit is phosphorylated by cdc2 kinase which regulates the G2 to M-phase transition.

Insulin stimulates protein synthesis by regulating the phosphorylation of translational factors, including EF-1 and EF-2. Binding of insulin to one of its receptors, IRS-1, causes autophosphorylation of the receptor and promotes the binding of phosphatidylinositol 3-kinase (PI 3-kinase) to the receptor. The association activates the kinase, leading to the phosphorylation and inactivation of EF-2 kinase.

Protein synthesis is increased in cardiac hypertrophy, and angiotensin II (AN II) has been shown to induce hypertrophy. Recently, it has been shown that AN II activates EF-2 by PP-2A-mediated dephosphorylation via a process that involves both PI 3-kinase and MAPK.

(2) Translational control of TOP mRNA
5'TOP (5' terminal oligo pyrimidine tract) is found in the 5' terminal region of vertebrate mRNAs, which encode abundant and key components of the translational apparatus, such as ribosomal proteins EF-1α, and EF-2. The mitogen-stimulated phosphorylation of ribosomal S6 protein leads to the preferential translation of TOP mRNAs. Conversely, inhibition of p70 ribosomal protein S6 kinase by the immunosuppressant macrolide rapamycin selectively suppresses mitogen-induced translation of TOP mRNA.

These mRNAs include some immunoglobulin mRNAs that direct the synthesis of antibodies.
against explanted tissues, and treatment with rapamycin allows the transplantation of immunologically different tissues from other individuals.

Insulin also stimulates the phosphorylation of ribosomal protein S6 and the EF-1α, β and δ subunits by a multipotential S6 kinase. The phosphorylation leads to the stimulation of poly(U)-dependent polyphenylalanine synthesis. Since the TOP includes an oligo(U) sequence, phosphorylated S6 is thought to stimulate polyphenylalanine synthesis by the same mechanism that mediates the preferential translation of TOP mRNAs.

Leucine also stimulates the phosphorylation of S6, resulting in the stimulation of EF-1α synthesis. As the stimulatory effects of leucine are inhibited by rapamycin which inhibits the protein kinase mTOR (mammalian target of rapamycin), it is thought that leucine activates mTOR which in turn activates S6 kinase. Although it remains unknown whether EF-1α is involved, S6 kinase has recently been shown to be a regulator of cell size in Drosophila: S6 kinase deficiency caused an extreme delay in development and a severe reduction in body size.

(3) Other modifications of EF-1

Although the functions remain unknown, various modifications of EF-1α have been reported, including methylation, acetylation, and myristoylation. Mammalian EF-1α has been shown to contain unique glyceryl-phosphorylethanolamine residues. Anchoring of EF-1α by phosphatidylinositol at the endoplasmic reticulum membrane has also been reported. In the case of EF-Tu, methylation attenuates GTP hydrolysis and is thought to increase translational accuracy. Recently, methylsterification of yeast EF-1α at the C-terminal lysine has been detected. As the methylster turns over more rapidly (half-life of less than 10 min) than the ordinal N-methyl group of lysine (half-life of over 4 h), it is expected that the former modification has some physiological function.

5. Signal Transduction and Elongation Factors

In addition to signal transduction related to translational control, EF-1α has been shown to participate in various other types of signal transductions in cellular processes such as cell growth, stress responses, and motility (Section 11).

(1) Phosphoinositide pathway and EF-1α

The phosphoinositide pathway is one of the major signal transduction cascades which is activated by growth factors. Interestingly, several enzymes in the pathway, such as phosphatidylinositol 4-kinase (PI 4-kinase), PI 4P-kinase, diacylglycerol kinase, and phospholipase C (PLC) have been shown to be associated with the cytoskeleton. Furthermore, PIK-A49, similar to EF-1α, has been shown to activate the PI 4-kinase-dependent increase of the bundling of F-actin (Fig. 2(A)). Collectively, these data indicate that many components of signal transduction are mobilized to actin filaments (F-actin), probably for the efficient relay or cross-talk of various signals.

More recently, interaction between phospholipase PLCγ and EF-1α has been demonstrated using a yeast two-hybrid system. In vitro analysis showed that the Src homology domains (SH2 and SH3) of PLCγ and the carboxyl terminal region of EF-1α are involved in the interaction. Their interaction in vivo has also been demonstrated by immunoprecipitation using anti-EF-1α antibody. As the interaction between EF-1α and PLCγ1 is increased by EGF treatment, EF-1α is thought to play a role in PLCγ-mediated signal transduction.

(2) Calcium signaling pathway and elongation factors

In the calcium signaling pathway, calmodulin delivers a calcium signal to a calmodulin-dependent kinase, resulting in the phosphorylation of some target proteins, including EF-1α.

The interaction of lily chimeric calcium/calmodulin-dependent protein kinase (CCaMK) with a homolog of EF-1α has been reported. The kinase catalyzes calcium- and calmodulin-dependent phosphorylation of EF-1α at the threonine residues which are located in the putative aa-tRNA binding site. The fact that the phosphorylation of EF-1α is Ca2+-dependent and that phosphorylated EF-1α has reduced actin-bundling activity, suggests the biological functions of CCaMK-dependent phosphorylation of EF-1α are in the calcium signaling pathway. The kinase is encoded by a single-copy gene in the lily and has no known animal homolog, in contrast to the calcium-dependent but calmodulin-independent ubiquitous CDP kinase that phosphorylates different sites of EF-1α. Phosphorylation of EF-1α by CDP kinase has been reported to be crucial for its function as an activator of PI 4-kinase.

Even the degradation of EF-1α itself is likely to be regulated by calcium, depending on the pH of cells. Drosophila calpain (calcium-activated neutral protease) has been shown to degrade the L5, L7, and L8 ribosomal proteins and EF-1α.

EF-2 is phosphorylated by a specific Ca2+/calmodulin-dependent protein kinase (EF-2 kinase) causing its complete inactivation. Insulin suppresses the kinase activity, leading to the reactivation of EF-2 by dephosphorylation by protein phosphatase PP-2A. The tumor promoter okadaic acid, which inhibits protein phosphatase activity, inhibits reticulocyte-lsate protein synthesis by increasing the net phosphorylation of EF-2.
Moonlighting Functions of Polypeptide Elongation Factor 1

(A) Actin filaments

- PI-4 kinase
- Myosin binding subunit
- Rho-associated kinase
- Myosin light chain

(B) Bundling of actin filaments

- Rho-associated kinase
- Control of stress fibers
- Severing of microtubules

(C) Control of stress fibers

- Severing of microtubules

(D) Repression of HIV-1 RNA replication?

- Repression of cellular mRNA expression

(E) Repression of HIV-1 RNA replication?

- Transcriptional control?

Fig. 2. Notable Interactions among EF-1 Subunits and Cellular Components.

(A) Control of cytoskeletal organization, PI: phosphatidylinositol, MLC: myosin light chain, (B) control of HIV-1 expression? (C) nuclear functions of EF-1α, VCCn: vigilin core complex in nucleus, VCCc: cytoplasmic VCC, (D) Signal transduction in apoptosis, (E) viral RNA synthesis.

6. Diseases and Elongation Factors

Recently, serious diseases such as cancer, systemic lupus erythematosus, and adult atopic dermatitis have been shown to be associated with changes in elongation factors. Elongation factors alone may not
be the principle causes of these diseases, but some diseases are likely associated with the dysfunction of elongation factors.

(1) Diphtheria and EF-2

Diphtheria toxin, which causes diphtheria in humans by inhibiting protein synthesis, is a 58-kDa protein secreted by lysogenic strains of Corynebacterium diphtheriae. The N-terminal B fragment domain of the toxin binds to a cell-surface receptor and is cleaved to form the B and A fragments, the former of which mediates the transport of the latter, the catalytic fragment, into the cell. The A fragment catalyzes the ADP-ribosylation of EF-2 at a modified histidine residue (His 715 in mammalian EF-2) called diphthamide, which is ubiquitous among eukaryotic EF-2s. The ribosylation leads to a complete loss of EF-2 activity and subsequent apoptosis.

As it is unthinkable that the diphthamide exists just waiting for diphtheria toxin, and as yeast do not suffer from diphtheria, the modified residue certainly has some physiological functions. There have been some reports suggesting the existence of cellular ADP-transribosylase: in polovina virus-transformed baby hamster kidney cells, the same peptide that can be ADP-ribosylated by diphtheria toxin has also been shown to be modified, and higher ribosylation was observed at 2% than 10% serum in the culture medium. These results suggest that ADP-ribosylation would control EF-2 activity under nutritional deprivation. However, the enzymes that transfer or remove the ADP moiety from EF-2 have not yet been isolated. The fact that ADP-ribosylated EF-2 has a long half-life in the cell also suggests the absence of a system that reverses the inhibition of EF-2 activity.

As EF-2 mutants deficient in diphthamide formation show temperature-sensitive cell growth, one of the suggested functions of diphthamide is to confer heat resistance on EF-2.

(2) Autoimmune diseases and translational factors

Adult atopic dermatitis (AAD) has been increasing recently in Japan. The causes of this disease are diverse, and include house dust, mites, sunlight, sweat, and stress. Recently, an anti-EF-1α autoantibody was shown to be elevated in AAD patients. It is thought that UV, scratching, and dermatitis injure the superficial lesional keratinocytes, and expose cytoplasmic EF-1α to the immune system, which stimulates a vicious cycle of exacerbation of the disease.

Systemic lupus erythematosus is an autoimmune disease in which anti-dsDNA autoantibody is formed. The antibody has been shown to react with EF-2, and inhibits in vitro translation. As the inhibition is partially overcome by EF-2 and abrogated by dsDNA, the cross-reaction between anti-dsDNA antibodies and EF-2 is specific and is thought to lead to cellular dysfunction.

Although the identification and cloning of relevant autoantibodies and autoantigens in human autoimmune diseases have been difficult, a phage display method and antigen expression libraries have been developed to address these problems. This method was used for the assay of antigens in serum from donors suffering from Felty's syndrome (neutropenia), and the anti-EF-1α antibody level was found to be elevated in 66% of patients.

Autoantibodies to ribosomal proteins P, S10, L12, and even to ribosomal RNA, have been detected. The anti-rRNA antibody has been used to detect the functional site of the GTPase-associated center within the 28S ribosomal RNA. The fact that the antibody blocks the bindings of EF-1 and EF-2 to the RNA supports the idea that both elongation factors interact alternately with the center in the process of translation. Anti-mRNP (mRNA protein complex) antibodies have been detected in rheumatic diseases. Even the antibodies to antibodies (glycated IgG) are formed in this disease.

(3) Alzheimer's disease and EF-2

In Alzheimer's disease, hyperphosphorylation of EF-2, which leads to inactivation of its translocation activity, has been observed. Moreover, polysomes isolated from Alzheimer's disease tissue have reduced activity. However, it is unknown whether phosphorylation and the repression of protein synthesis are causes or results of the disease.

(4) Diabetes and elongation factors

Protein synthesis is decreased in type 1 and 2 diabetes. Recently, unbalanced expression of EF-1α mRNA (2- to 6-fold overexpressed) compared with EF-1β and γ mRNAs has been observed in type 1 and 2 diabetic skeletal muscle. Similar results have also been obtained in animal models of type 1 diabetes. EF-1α but not EF-1β expression is induced in streptozotocin-induced diabetic rats, and this effect is reversed by insulin treatment. Although the mechanism of the unbalanced regulation of the expression of EF-1 subunits in diabetes is unknown, one possibility is that the expression of EF-1α may be up-regulated to compensate for reduced protein synthesis in diabetes. In cardiac muscle of experimentally induced diabetic rats, decreases in translational efficiency and ribosome and EF-2 content have been observed. The fact that insulin therapy increased the EF-2 content of diabetic rats to control values suggests that the decreased EF-2 content at least partially accounts for the impaired rate of translation in diabetes. As insulin also controls the phosphorylation of EF-1, EF-2, and ribosomal protein 6S (Section 4), further studies will be needed to clarify the relationship between diabetes and elongation factors.
(5) Cancer and elongation factors

In human pancreas, colon, breast, lung, and gastric tumors, EF-1α mRNA is overexpressed. In metastatic rat mammary adenocarcinoma, EF-1α is also overexpressed. The intracellular distributions of F-actin and EF-1α are highly correlated, and it is thought that actin-containing cytoskeletal structures are important for supporting the cellular motility required for metastasis. EF-1α mRNA is overexpressed in gastrointestinal carcinoma, colorectal adenoma, and colorectal carcinoma. Moreover, EF-1δ expression is modulated by oncogenes in human epithelial cells.

Normal cells in which EF-1α was overexpressed became highly susceptible to oncogenic transformation by 3-methylcholanthrene and ultraviolet light. Surprisingly, human prostatic carcinoma oncogene product PTI-1 has been shown to be similar in sequence to EF-1α truncated in the N-terminal residues, the mRNA of which bears a 5'-UTR with significant similarity to the Mycoplasma hyopneumonia 23S rRNA. Furthermore, the expression of a cDNA clone containing the PTI-1 coding region in nude mice led to tumor formation, and that expression of antisense PTI-1 RNA reversed the cancer phenotype.

The phenotypes of various cancers are related to growth factors and their receptors. Quite recently, the remarkable findings that an EGF-like nerve cell growth factor, hereglin-β (HRG), induces EF-1α in proliferating cells, including cancer cells and that antibodies to the growth factor receptor suppress the expression of EF-1α and proliferation were reported. Interestingly, one of the potent Sp1 sites of the EF-1α promoter was shown to be concerned with the growth-factor-stimulated expression of EF-1α. Mutants or inhibitors of the MAP kinase cascade (p38 MAPK and MEK), but not PI 3-kinase, suppressed the HRG-induced stimulation of EF-1α promoter activity. Furthermore, acetylation of histone H3 and H4 was shown to be associated with the HRG-dependent activation of the EF-1α promoter Sp1 site. Although the function of the overexpression of EF-1α in rapidly proliferating cancer cells remains unknown, three possibilities have been suggested: (i) stimulation of translation of growth-related proteins, (ii) reorganization of the cytoskeleton, (iii) yet undefined roles in the nucleus, as EF-1α has the ability to form a complex with ZPR1, which is thought to be a transcription factor. Extensive reviews of "translation and cancer" have been published.

7. Apoptosis and EF-1α

"Apoptosis" is programmed cell death. Findings that suggest a relationship between apoptosis and EF-1α have been accumulating. In Trypanosoma cruzi, nuclear localization of EF-1α has been observed in parasites undergoing apoptosis. EF-1δ binds with HIV Tat, which is known to induce apoptosis, and shutoff host cell mRNA translation (Fig. 2(B)). Furthermore, various signals, including oxidative stress and nutritional deprivation, which lead to apoptosis, regulate the expression of both p53 and EF-1α.

(1) Up-regulation of EF-1α gene by p53

p53 is a major tumor-suppressor gene product that has transcriptional factor activity, and mutations of the p53 gene are the most frequently observed mutations in human cancers. p53 accelerates apoptosis, and consequently suppresses tumorigenesis. p53 is thought to monitor the integrity of DNA in G1 phase. When the damage in DNA is repairable, p53 induces p21 and other proteins to repair the damage. When the damage is too severe to repair, p53 directs apoptosis to proceed.

An erythroleukemic cell line bearing only a temperature-sensitive mutant p53 underwent apoptosis at 32°C, but not at 37°C. Using these cells, genes up-regulated by p53 were isolated by the mRNA differential display method. One of the genes expressed at a level more than two-fold higher at 32°C than at 37°C was identified as the EF-1α gene. Furthermore, a p53-responsive element, RRRCWWGYYY, which confers the capacity for induction by p53 on some genes, is conserved in the human, rat, and frog EF-1α genes. When the erythroleukemic cells described above were transfected with a plasmid containing the p53-responsive element and the luciferase gene, the expression of luciferase activity increased about two-fold upon a temperature shift to 32°C. As EF-1α has microtubule-severing activity, and microtubules become distorted at 32°C, it is speculated that microtubule-severing resulting from up-regulation of EF-1α by p53 is one of the causes of apoptosis.

In mouse 3T3 fibroblasts, increased expression of EF-1α confers increased susceptibility to serum-deprivation-induced apoptosis. Furthermore, the suppression of EF-1α expression by antisense EF-1α RNA prevents the induction of apoptosis by serum deprivation. Based on these findings, it has been suggested that when EF-1α is abundant the cells are in a proapoptotic mode, and conversely, when EF-1α is scarce, the cells are in an antiapoptotic mode.

(2) Oxidative Stress and Apoptosis

Induction of p53 in oxidative stress-induced apoptosis has been observed in cardiomyocytes. In cells undergoing hydrogen peroxide-induced apoptosis, EF-1α expression is induced in proportion to the concentration of hydrogen peroxide. Interestingly, the induction of EF-1α is most likely controlled at the translational level, as the level of EF-1α mRNA is not changed by hydrogen peroxide treatment, and cyclo-
heximide, but not actinomycin D, inhibits the induction. Furthermore, the repression of EF-1α expression by a transient transduction of antisense EF-1α cDNA prevents the induction of apoptosis by oxidative stress in proportion to the reduction of the EF-1α level. The EF-1α level itself, rather than its protein-synthesizing activity, appears to be more important in the execution of the apoptotic program.\textsuperscript{103}

From this circumstantial evidence, the roles of p53 and EF-1α in apoptosis can be characterized as follows: “To be or not to be, that is not the question; rather, the execution is conducted by p53 and EF-1α.” More extensive work will be needed, however, to clarify the functions of elongation factors in apoptosis. It will be important to know the relationship between the postulated EF-1α-mediated pathway and well-established apoptotic pathways such as that from p53 to Bcl-2, which leads to activation of caspases and caspase-activated DNase (CAD).\textsuperscript{104,105}

8. Life Cycle and Elongation Factors

1. Senescence and elongation factors

Rapidly proliferating cells, including cancer cells, have higher protein-synthesizing activity and a higher content of elongation factors, while senescent animals and cultured cells have a reduced rate of protein synthesis, for which reduced expression or activity of EF-1α and EF-2 has been thought to be responsible. However, careful interpretation of the results should be done, since there are several EF-1α genes, and specific EF-1α species that have specific functions may be expressed independently, and moreover, EF-1α and EF-2 activities can be regulated by various modifications depending on the needs of specific cells. For example, EF-1α was reduced in senescent human skeletal muscle and was thus thought to be responsible for the reduction of protein synthesis in senescence. However, it has been shown more recently that the total protein and mRNA levels of EF-1α and the EF-1α homologue S1 are not different between younger and older muscle.\textsuperscript{106}

2. Longevity and elongation factors

Transgenic Drosophila melanogaster with an additional copy of the EF-1α gene were reported to have an extended life span.\textsuperscript{107} In other experiments, however, the lifespan of virgin males was not extended by the introduction of the EF-1α gene. The extension of life span observed in fames may be in part the consequence of reduced fecundity.\textsuperscript{108}

In Caenorhabditis elegans, mutations that reduce the P1 3-kinase (daf-23) or insulin-like receptor (daf-2) favor entry into the dauer state during larval development and extend the lifespan in adults.\textsuperscript{109,110} As the insulin signal via P1 3-kinase activates EF-2 by dephosphorylation of inactive phosphorylated EF-2 and stimulates cell proliferation, saving of energy or reduced cellular activities in the dauer state due to the mutation may lead to the extended lifespan. It is thought that the dauer state is the normal state in the process of evolution in which whole animals are often exposed to nutritional deficiency. In the present age of repletion, the dauer-related genes such as daf-2 and daf-23 are likely at capacity, especially in type II diabetes, resulting in an increased incidence of diabetes. These data indicate that EF-2 is probably involved in the control of the signal transduction pathways to longevity. The molecular basis of the Japanese proverb “To be moderate in eating is to live longer (Feed by measure and defy the physician)” will be revealed by further analyses of the function of elongation factors.

9. Life Cycles of Viruses and Elongation Factors

1. Participation of elongation factors in virus replication

As early as 1972, host-encoded EF-Tu, EF-Ts, and ribosomal protein S1 were shown to be components of Qβ phage RNA replicase.\textsuperscript{112} As the Qβ phage RNA and the related family of RNAs have a tRNA-like structure at the 3’ terminal end and GTP is required for the replication of the RNA, EF-Tu is thought to be necessary for the binding of the replicase to the phage RNA. This discovery about Qβ phage encouraged us to expect the participation of eukaryotic elongation factors in virus RNA replication in eukaryotic cells. More than three decades after the analogous discovery in Qβ, an association between EF-1\textit{α}βγ and the RNA polymerase of vesicular stomatitis virus (a negative-strand RNA virus) necessary for the full activity of positive-strand RNA synthesis, was recently reported (Fig. 2(E)).\textsuperscript{113}

An interaction between EF-1α and the 3’ stem-loop region of West Nile virus (WNV) genomic RNA has also been reported.\textsuperscript{114} This interaction is sequence-specific, and the dissociation constant for the complex has been calculated to be as low as 1.1 \( \times \) 10\(^{-9}\) M. Although the function of the interaction is unknown, dephosphorylation of EF-1α inhibits its binding to WNV 3’ stem-loop RNA. The 3’ stem-loop of dengue virus RNA is required for virus replication.\textsuperscript{115} Based on these data, it has been speculated that EF-1α participates in the replication of WNV RNA.

Plant virus RNAs such as brome mosaic virus and turnip yellow mosaic virus RNAs have a tRNA-like cloverleaf structure at the 3’ terminus. The structure can be recognized by valyl-tRNA synthetase and valylated at the 3’ terminus. Furthermore, the valyl-tRNA-like structure associates with EF-1α with an affinity similar to that of val-tRNA.\textsuperscript{116} Recently, a 5’ cloverleaf structure in poliovirus RNA (positive-strand) has been shown to be a cis-acting replication element required for negative-strand synthesis. That
is, mutant RNA in which the cloverleaf was disrupted and a cap-structure was introduced into the 5' end of the RNA directed translation efficiently, but not negative-strand RNA synthesis.\(^\text{106}\)

(2) Participation of EF-1 subunits in the control of HIV or HSV-1 expression

Recently, HIV-1 Gag polyprotein, which has key functions at almost all stages of the viral life cycle, was shown to interact with EF-1\(\alpha\). The interaction requires tRNA, and interestingly, EF-1\(\alpha\) has been thought to contribute to tRNA incorporation into HIV-1 virions.\(^\text{117}\) Furthermore, Gag associates with F-actin and has been suggested to play an important role in HIV-1 assembly (Fig. 2(B)).\(^\text{118}\)

HIV Tat protein is an early regulatory protein which is critical for HIV gene expression and replication. EF-1\(\delta\) has been shown to interact with the second coding exon of Tat, and is thought to repress HIV-1 RNA replication (Fig. 2(B)). This interaction reduces the translational efficiency of cellular but not viral mRNAs.\(^\text{117}\) This is the first evidence for the participation of factors similar to EF-1\(\beta\) in the selective translation of mRNA. Viral proteins that may regulate EF-1\(\delta\) are also detected in herpes simplex virus 1 (HSV-1). Infected cell protein No. 0 of the virus binds EF-1\(\delta\).\(^\text{119}\) Another viral protein (UL13 protein kinase) hyper-phosphorylates EF-1\(\alpha\).\(^\text{120}\)

(3) EF-1\(\alpha\) homolog antiviral protein SKi7p

The superkiller SKi7 gene negatively controls the copy number of yeast double-stranded L-A and M RNA virus. When propagated in yeast with an SKi7 mutation, M virus produces killer toxin and kills other yeasts. The 747-amino-acid protein SKi7p is similar to EF-1\(\alpha\) and has been suggested to have translation regulatory activity. SKi7p represses the expression of non-poly (A) mRNA, but not poly(A) mRNA, and inhibits the propagation of yeast viruses.\(^\text{121}\) The mechanism of the repression by SKi7p remains unknown.

10. Cold-response and Elongation Factors

Correlations between cold-acclimation and higher levels of expression of an EF-1\(\alpha\) with a single base-mutation have been reported in winter barley.\(^\text{122}\) Although the mutation is located in a motif involved in ensuring the fidelity of translation,\(^\text{123,124}\) the relation between the cold-acclimation and translational fidelity is unknown. A low-temperature-induced EF-1\(\alpha\) has also been isolated from maize. In the leaves, the EF-1\(\alpha\) mRNA level is increased at 5°C, whereas in roots the overall EF-1\(\alpha\) mRNA level is transiently decreased and subsequently increased. These results indicate that the expression of EF-1\(\alpha\) is regulated differently in leaves and roots under cold stress.\(^\text{125}\)

In cold acclimated rainbow trout, elongation factor activities have been improved.\(^\text{126}\) Using hybrid protein synthetic systems (rat and Antarctic fish), EF-1\(\alpha\) has been suggested to confer poikilothermy on mammals.\(^\text{127}\)

Recently, the structure of EF-G from the low-temperature-adapted bacterium \textit{Arthrobacter globiformis} S155 has been analyzed, and the protein strategy for maintaining its flexibility under cold conditions by reducing intramolecular salt bridges has been described.\(^\text{128}\) Although the mechanism is unknown, a cold-sensitive mutant of \textit{Saccharomyces cerevisiae} bearing a \textit{drs2} mutation, in which the processing of 20S precursor to mature 18S rRNA is slowed, can be rescued by extra copies of the EF-1\(\gamma\) gene.\(^\text{129}\)

11. Cytoskeletal Organization and Elongation Factors

In the last decade, remarkable observations showing the association of elongation factors with cellular structures including the cytoskeleton, centrosphere,\(^\text{130}\) and mitotic apparatus have reported. These novel activities of elongation factors have been thought to be closely related to important cellular processes such as apoptosis, carcinogenesis, and signal transduction. Furthermore, cytoskeletal structures function as sites for efficient and targeted protein synthesis.\(^\text{131}\)

(1) Actin bundling activity of EF-1\(\alpha\)

EF-1\(\alpha\) is a ubiquitous and abundant protein. Large increases in the EF-1\(\alpha\) mRNA level are observed in rapidly proliferating cells, such as cultured cells, embryonic cells and cancer cells. Even in normal growing cells, the molar ratio of EF-1\(\alpha\) to other translational factors such as EF-2 and ribosomes is nearly 20 to 30.\(^\text{130}\) Although the reason for the high content of EF-1\(\alpha\) remained unknown for more than two decades, a breakthrough in answering this question had been brought about by the discovery of the localization of EF-1\(\alpha\) on F-actin. That is, one of the actin binding proteins (ABP-50) isolated from \textit{Dictyostelium discoideum} was identified as EF-1\(\alpha\).\(^\text{132}\) Furthermore, ABP-50 was shown to bundle F-actin during chemotaxis.\(^\text{121}\) These are the first clear indications of the interaction between the cytoskeleton and the translational machinery. EF-1\(\alpha\) is also an activator of a PI 4-kinase that binds actin and increases actin bundling.\(^\text{62}\) The actin bundling activity of EF-1\(\alpha\) has been extensively analyzed \textit{in vitro} using purified factors from plants and animals. \textit{Tetrahymena} EF-1\(\alpha\) induces bundling of rabbit skeletal muscle F-actin as well as its own F-actin. Immunoprecipitation experiments showed that the binding ratio of \textit{Tetrahymena} EF-1\(\alpha\) to skeletal muscle F-actin in the bundles is 1:1.\(^\text{133}\)

Binding of EF-2 with F-actin (about 0.12 mole per mole of actin monomer) has been reported, suggest-
ing a possible link between the protein synthetic machinery and the cytoskeleton.\textsuperscript{136}

(2) \textit{F-actin and protein synthesizing activity}

The reasons why the activity of the \textit{in vitro} protein synthesizing systems is much lower than that of intact cells have remained unknown. One of the reasons is thought to involve the higher-ordered organization of the translational apparatus as follows. The protein-synthesizing activity of CHO cells permeabilized by saponin is 40\% of that of the intact cells, while the cell-free protein synthesizing activity is about 1\%.\textsuperscript{45} To assay these activity levels, the intact and permeabilized cells were both treated at 0\(^\circ\)C at which temperature the protein-synthesizing activity of these cells drops to about 5\% of the level at 28\(^\circ\)C. Under these conditions, F-actin is disrupted. However, when both types of cell were treated at room temperature in the presence of glucose, both the integrity of the F-actin and the protein-synthesizing activity were maintained. These results suggest that F-actin is necessary for efficient protein synthesis.

During the healing of wounded potato tubers, F-actin is organized around the wound, and the translational apparatus is gathered at the filaments for efficient protein synthesis for the healing of the wound.\textsuperscript{135} A higher plant extracellular vitronectin-like adhesion protein, PVNI, from NaCl-adapted tobacco cells, is almost identical with EF-1\(\alpha\). PVNI is shown to be localized in the cell wall of cortical and transmitting tissue cells of pollinated mature styles. Isolated PVNI promotes the spreading of cultured baby hamster kidney cells on the surface of microtiter wells.\textsuperscript{136} However, it is not clear whether PVNI functions physiologically as an adhesion protein in plants.

(3) \textit{Microtubule organizing activity of EF-1\(\alpha\)}

Some factors that sever microtubules have been thought to be involved in microtubule reorganization during the cell cycle. In 1994, a 48-kDa microtubule-severing protein was purified from \textit{Xenopus} eggs and identified as EF-1\(\alpha\) (Fig. 2(A)).\textsuperscript{137} Furthermore, human EF-1\(\alpha\) expressed in \textit{E. coli} has been shown to have microtubule-severing activity \textit{in vitro}. The microinjection of the protein into fibroblasts induces rapid and transient fragmentation of cytoplasmic microtubule arrays.\textsuperscript{137}

Furthermore, calcium/calmodulin has been shown to modulate the microtubule-bundling activity of a carrot EF-1\(\alpha\)-like factor.\textsuperscript{138} EF-1\(\alpha\) has also been shown to stabilize microtubules by reducing the shortening velocity of the filaments.\textsuperscript{139} Recently, two putative microtubule-binding domains were detected on EF-1\(\alpha\). One is located in the N-terminal domain I, and the other is in the C-terminal domain III, and they are involved in the conditional and unconditional binding of EF-1\(\alpha\) to microtubules, respectively.\textsuperscript{140} The mechanisms of controlling or switching between the EF-1\(\alpha\)-dependent microtubule severing and bundling activities, however, are still unknown.

(4) \textit{Regulation of the organization of the cytoskeleton by Rho-associated kinase and EF-1\(\alpha\)}

The small GTPase Rho family has some of the most important signal transducing proteins,\textsuperscript{141} and mediates various signals leading to chemotaxis, gastrulation, smooth muscle contraction, cell-cell adhesion, membrane ruffling, regulation of F-actins organization into stress fibers, and so on. In these pathways, Rho activates Rho-associated kinase (Rho-kinase), and the kinase directly phosphorylates myosin light chain, the myosin-binding subunit of myosin phosphatase, and adducin, and thereby regulates the stress fiber formation and cellular movement.

Recently, recombinant EF-1\(\alpha\) has been shown to be phosphorylated by Rho-kinase in a reaction dependent on the presence of Rho and GTP (GTP\(\gamma\)S) \textit{in vitro}. The binding activity of EF-1\(\alpha\) to F-actin is decreased by the phosphorylation, as is the actin bundling activity of EF-1\(\alpha\) (Fig. 2(A)).\textsuperscript{142} As EF-1\(\alpha\) binds to the myosin binding subunit of myosin phosphatase, it has been suggested that both Rho-kinase and myosin phosphatase regulate the phosphorylation state of EF-1\(\alpha\), which in turn regulates the organization of the actin cytoskeleton.\textsuperscript{142}

12. Proteasome and Protein-disulfide Isomerase Functions of EF-1\(\alpha\) (EF-Tu)

EF-1\(\alpha\) and EF-Tu have been suggested to regulate 26S proteasome-dependent degradation of ubiquitin-conjugated proteins.\textsuperscript{143, 144} Although the observations and speculation that "protein synthesis and degradation may be regulated by a common factor, EF-1\(\alpha\)" are interesting, these data and ideas have not yet been confirmed.

EF-Tu also has a protein-disulfide isomerase activity and stimulates the refolding of randomly oxidized RNase. Based on these results, EF-Tu has been suggested to be an ancestral protein folding factor that appeared before dedicated chaperones and protein disulfide isomerases.\textsuperscript{145} It is unknown whether this activity has physiological meaning, and whether EF-1\(\alpha\) has the activity.

13. Specific EF-1\(\alpha\) for Selenocysteine Insertion

Selenium is an essential element for bacteria, achaena, and animals. Most of it, in the form of selenocysteine, is contained in some enzymes such as glutathione peroxidase and thioredoxin reductase, which maintain the redox states of the cell. Selenium
deficiency causes liver necrosis, muscular dystrophy, and so on. Selenocysteine has been called the 21st amino acid in protein. In prokaryotes, the amino acid is incorporated into seleno-proteins from selenocysteinyl-tRNA (sec-tRNA) and is encoded by a specific UGA termination code followed by a "stem loop structure" termed SECIS (selenocysteine insertion sequence) (Fig. 3(A)). The UGA is recognized by a specific EF-Tu called SelB that contains the binding sites for sec-tRNA and SECIS. Sec-tRNA is synthesized by sec-tRNA synthetase from seryl-tRNA and selenocysteine-specific EF-1α.

The existence of a factor that stabilizes the hydrolysis of sec-tRNA was suggested in 1994, and a factor termed mSelB (eEF-1sec), which has the activity to bind sec-tRNA but not other aa-tRNAs has been isolated. However, the factor lacks a recognition site for the more complicated eukaryotic SECIS, which is located in the 3' untranslated region (3' UTR) following the actual termination codon UAG (Fig. 3(B)).

A eukaryotic factor called SBP2 (SECIS-binding protein 2), which recognizes eukaryotic SECIS, has been discovered recently and shown to deliver sec-tRNA in the form of an eEF-1sec-SBP2 complex to the UGA codon preceding SECIS. The reason why eukaryotic SECIS is located far from the selenocysteine insertion site can be explained by the fact that there are several selenocysteine insertion sites in some eukaryotic selenoproteins. For example, the rat selenoprotein P open reading frame contains two SECISs and 10 UGA codons, which are used for the selenocysteine insertion. The flexibility of the RNA sequence between SECISs and selenocysteins insertion sites is thought to be important for the insertion. In contrast, in prokaryotes only one site per selenoprotein is known and the insertion site is governed by the presence of a nearby SECIS.

14. Nuclear Function of EF-1α

Interestingly, EF-1α has been shown to interact with a zinc finger protein, ZRP1, in proliferating cells. ZPR1 is located in the cytoplasm of quiescent mammalian cells, and treatment of cells with mitogens including epidermal growth factor (EGF) induces the interaction of ZPR1 with EF-1α and the redistribution of both proteins from the cytoplasm to the nucleus (Fig. 2). Mutational analysis of Saccharomyces cerevisiae showed that disruption of the binding of ZPR1 to EF-1α results in the accumulation of cells in the G2/M phase of the cell cycle and in defective growth. Furthermore, restoration of the interaction between ZPR1 and EF-1α restores normal growth. Although the function of ZPR1 in the nucleus has not yet been defined, it has been suggested that the protein may act as a signaling molecule that communicates mitogenic signals from the cytoplasm to the nucleus.

EF-1α is associated with the mitotic apparatus in sea urchin eggs. As EF-1α has in vitro α, β, and δ tubulin binding activity, it has been suggested that EF-1α participates in the nucleation of astal microtubules, and in subsequent mitotic spindle formation.

Recently, we analyzed the localization of various EF-1 subunit-GFP fusion proteins in tobacco BY-2 cells. At the interphase, all the subunits tested (EF-1α, β, and γ) were colocalized with cytoplasmic F-actin. As the fibrous EF-1 structures are disrupted by cytochalasin D, which depolymerizes F-actin, the EF-1 subunits are most likely associated with F-actin. Upon cold treatment, the fibrous structures of EF-1α or EF-1γ disappear, and are subsequently reorganized upon incubation at 25°C for several minutes in the presence of glucose. At mitosis, EF-1 subunits and F-actin are associated with mitotic spindles. Further analyses of the localization and functions of EF-1 subunits will provide new insights into cell biology.

Surprisingly, it has been shown that the integrity of the structure and function of tRNA synthesized in the nucleus is inspected before its transport to the cytoplasm. The export is thought to be coupled

Fig. 3. The Mechanisms of Selenocysteine Insertion in Prokaryotes (A) and Eukaryotes (B).

(A) bSelB: bacterial selenocysteine specific EF-Tu, SECIS: selenocysteine insertion sequence, mSelB: mammalian selenocysteine-specific EF-1α, SBP: SECIS binding protein.
with a ubiquitous nuclear ribonucleo-protein K-like protein called vigilin. When recombinant vigilin or immuno-affinity-purified nuclear vigilin core complex (VCC<sub>r</sub>), which contains EF-1α and exportin-t, is microinjected into human cells, tRNA export from the nucleus is accelerated. EF-1βγ, in contrast, has been detected only in the cytoplasmic complex VCC<sub>c</sub> (Fig. 2(C)).<sup>156</sup>

15. Practical Uses of Elongation Factors or the Molecules Related to the Functions of Elongation Factors

As elongation factors are not only key factors in translation, but also the most prominent multifunctional proteins, there have been and will continue to be numerous applications of them in various fields. Among them I will discuss, only several themes of current interest.

(1) Application of EF-1α promoter

As the EF-1α promoter is one of the strongest promoters in the cell (Section 3), it has been used for the cellular expression or for the industrial production of some proteins. In Aspergillus oryzae, the EF-1α gene promoter of the same species has been used for the expression of the polygalacturonase gene in high yield (100 mg/l).<sup>157</sup>

pEF-LAC is an inducible high-level expression vector that contains the human EF-1α promoter and three lactate operator sequences. This vector can be used for the controlled expression of various cloned genes in a variety of mammalian cells.<sup>158</sup> A vector containing one of the two medaka (a fish) EF-1α promoters and a green fluorescent protein GFP gene, EF-1α-A-GFP, has been microinjected into fertilized medaka eggs and transgenic medaka have thereby been produced.<sup>159</sup> As all the cells of the fish expressed the GFP gene highly, this is a useful model fish for a cell or nuclear explantation.

EF-1α promoter has also been used for establishing tetracycline-inducible stable linens of human cancer cells with consistent expression.<sup>160</sup> For studies of hepatitis C virus (HCV) pathogenesis, cell lines expressing the HCV core protein have been established by using the EF-1α promoter.<sup>161</sup>

(2) Molecular markers of disease or injury

As described in Section 6, EF-1α and EF-1γ are overexpressed in various cancers, and these factors will be useful as molecular markers of some cancers. As there is a strong correlation between the level of EF-1γ and malignancy, information on the level of EF-1γ facilitates the preoperative decision-making.<sup>162</sup> The unique oncoprotein of prostate cancer PTI-1, which contains a truncated EF-1α sequence (Section 6), has been amplified by RACE and used as a marker of PTI-1-dependent prostate, breast, colon, and lung carcinomas.<sup>163</sup> This method is so sensitive that carcinoma cells in the bloodstream of patients with metastatic prostate cancer has been detected, and it has been suggested that this gene will provide a sensitive and specific monitor of prostate cancer progression as reflected by the presence of cancer cells in a patient's bloodstream. As antisense inhibition of the PTI-1 oncogene reverses cancer phenotype,<sup>164</sup> the gene is thought to serve as a target for the gene-based therapy of prostate and other cancers.

The rat EF-1α homolog S1, which is expressed only in brain, heart, and muscle, can be used as a marker of injury-elicited regeneration. In muscle, the ratio of EF-1α/S1 is high during the early embryonic stage. The ratio becomes low by postnatal day 14, and the low ratio is low in adulthood. Interestingly, the ratio becomes high upon muscle injury, and returns to the original low value when the injury is repaired by regeneration. These results can be used for the molecular marker for injured muscle and its repair.<sup>164</sup>

Reactive oxygen species (ROS) induce apoptosis in cardiomyocytes, and have been thought to be one of the causes of conductance disturbances and cardiomyopathies, including arhythymogenic right ventricular dysphasia, diluted cardiomyopathy, and so on.<sup>99</sup> Since ROS induce expression of p53 and EF-1α, these molecules will be useful as molecular markers and for the future development of therapeutic methods for heart diseases.

(3) Cancer and AIDS therapies

Anti-growth factor receptor antibodies suppress both growth-factor-dependent expression of EF-1α and the proliferation of cancer cells (Section 6). In the U.S.A., antibodies to growth factor receptors are being widely tested in clinical trials for cancer immunotherapy. A humanized antibody to EGF receptor C225 is now in phase II clinical trials.<sup>165</sup> In combination with chemotherapy (cysplatin), an antibody to growth factor receptor HER2 is in phase II clinical trials.<sup>166</sup> As antisense inhibition of the PTI-1 oncogene reverses the cancer phenotype (Section 6),<sup>99</sup> the gene is thought to serve as a target for the gene-based therapy of prostate and other cancers.

As only one molecule of diphtheria toxin or Pseudomonas toxin A fragment is sufficient to kill a cell by inactivating EF-2 (Section 6), some chimeric molecules between toxins and ligands have been tested for therapeutic purposes: toxin A mutant-interleukin 13 for epithelial carcinomas<sup>167</sup> and toxin A fragment-CD4 for HIV.<sup>168</sup> Even a chimeric molecule between EGF receptor antibody and Pseudomonas toxin A is under development.<sup>169</sup> Because toxin A is so toxic, its certain delivery to the target cells alone will be critical. "Tat", which binds to EF-1α and participates in the control of the expression and replication of HIV (Section 9), is thought to be one of the
targets for future clinical therapy of AIDS.\textsuperscript{98} Analyses of the physiological significance and mechanisms of the interaction between EF-1 subunits and Tat will be important for the development of such therapy.

As one of the target sites for quercetin (saponin) in EF-1\textalpha , it has been supposed that plant inhibitors of protein biosynthesis might be useful as specific antitumor agents.\textsuperscript{169} As the plant toxin ricin, which has similar structure and function to O-157 verotoxin, eliminates the adenine nucleotide of 28S rRNA which is essential for the function of EF-1\textalpha and EF-2,\textsuperscript{170} the toxin is important not only for basic research, but also for the development of therapeutic methods for O-157 food poisoning and cancer.

(4) Nutritional improvement of cereals
The lysine content of the maize protein zein is low, and this is a serious nutritional problem when maize is used as a food for humans or monogastric domestic animals. However, a mutant called "opaque" has a higher lysine content, which correlates well with the EF-1\textalpha content.\textsuperscript{172,173} The expression of several EF-1\textalpha genes out of 10 to 15 EF-1\textalpha genes is increased in the endosperm of the opaque2 mutant. The lysine content of zein itself is not changed in the mutant, but the ratio of some lysine-rich proteins in seeds to zein is increased. As EF-1\textalpha, which has actin bundling activity, colocalizes with F-actin around protein bodies, it has been speculated that EF-1\textalpha stimulates the targeting of lysine-rich proteins to the endosperm through F-actin.\textsuperscript{174} However, use of the mutant as a crop is not practical at present, as the total protein content of the opaque mutant seeds is low.

Quantitative trait locus mapping of loci influencing the EF-1\textalpha content in maize endosperm is underway,\textsuperscript{175} and the elucidation of the mechanism of the correlation between the content of EF-1\textalpha and lysine will lead to the development of methods to improve the nutritional quality of some cereals including rice, which are deficient in lysine.

16. Epilogue

As discussed above, the development of research on elongation factors in the last half-century has revealed that EF-1 is one of the most prominent "supermultifunctional proteins". Further extensive and detailed exploration of the structures and functions of elongation factors, especially EF-1\textalpha, will lead to better understanding of fundamental strategies for living such as cell proliferation, cell division, apoptosis, stress responses, nutrition, and the coordination of these processes. Such studies will also lead to conquering some serious diseases related to elongation factors, including adult atopic dermatitis, AIDS, diabetes, and tumors. The accumulation of information on elongation factors will facilitate further improvement of cell-free protein synthesizing systems, which will be one of the most important bases of the postgenomic techniques termed the "proteomix." Thus, it can be said that research on protein biosynthesis centered on the moonlighting functions of elongation factors will be one of the most illuminating subjects of basic and applied bioscience in the new century.

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