THE EVOLUTION OF ELONGATION FACTORS Tu AND G BY GENE DUPLICATION

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1. Introduction

The evolution of protein synthesis has long been a source of speculation and remains one of the principal problems in the study of cellular evolution. Iskigami et al. [1] have recently proposed a model for the sequential development of a primitive ribosomal system which could have led to the present protein synthesis mechanism. One of the crucial steps in this process is the advent of the elongation factors with their numerous inherent advantages. The principal prokaryotic elongation factors are EF-Tu and EF-G. The primary function of EF-Tu is to bind aminoacyl tRNA and to direct it to the A site of the ribosome, while EF-G translocates the ribosome relative to the mRNA template [2]. EF-Tu and EF-G are similar in that they both show GTPase activity and interact with the ribosomal proteins L7/L12 [3]. Also, these proteins are located adjacent to each other in the λ*fus* 2 operon of *E. coli* [4]. In this report we show that EF-Tu and EF-G have several common structural features and that they probably arose from a common ancestor by gene duplication.

2. Methods

Calculations of α-helical and β-structures and of β-turns were made by the method of Chou and Fasman [5,6]. The amino acid sequence of EF-Tu fragment, Ala–Phe–Asp–Gln–Ile–Asp–Asn–Pro–Glu–Glu–Lys–Ala–Arg, was obtained from tryptic and chymotryptic peptides of cyanogen bromide peptide CB-1 [7] by methods described earlier [7,8].

3. Results and discussion

Alakhov et al. [9] have recently published the amino acid sequence of a large peptide which was obtained by limited trypsin cleavage of EF-G and which contains a cysteine residue located [cf. ref. 10] at the GTP binding site. Although Alakhov et al. [9] reported no homology between their peptide and a peptide from EF-Tu [8], a comparison with more recent data [7,11] shows clearly that there is homology between the two proteins.

Figure 1 shows schematically the two regions being compared. Although EF-G (MW 80 000) is nearly twice as large as EF-Tu (MW 44 000), both regions are located near the N-termini of the proteins. The cysteine residues at the GTP binding sites also occupy similar positions. The sequence data are presented in fig.2. By assuming a few insertion or deletion events, 33% homology was observed. When conservative substitutions are considered, 47% homology is seen. These calculations take into consideration a 10-residue deletion in EF-Tu at positions 23–32. It is interesting to note that regions containing proline (at positions 37, 47 and 78) are highly conserved.

The conformational parameters P_α, P_β, and P_t for amino acids in α-helixes, β-sheets and β-turns were calculated for regions of the two proteins according to Chou and Fasman [5,6]. Common β-structures are predicted for residues 15–20 and 67–73, as well as a β-turn at residues 46–49. A major difference is the absence of an α-helical region (residues 21–34) in EF-Tu.

Based on comparison of amino acid sequence and predicted secondary structure, it seems likely that the

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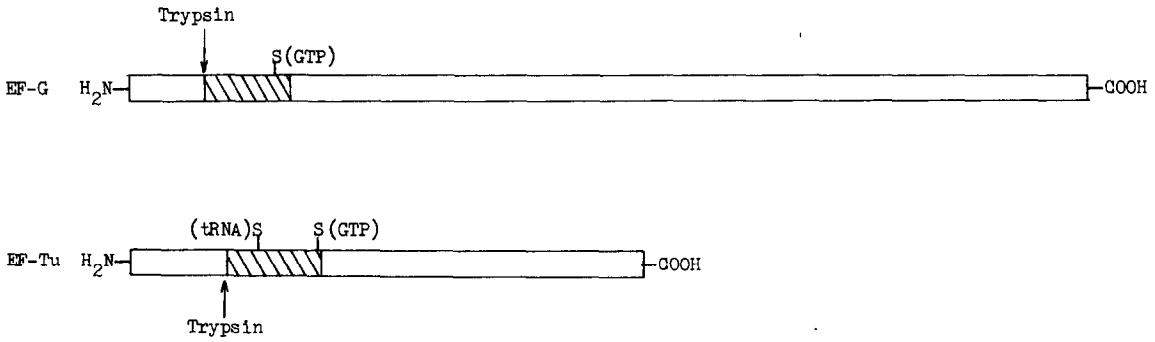

Fig.1. Schematic representation of EF-G and EF-Tu showing the relative sizes of the proteins and the locations of sulfhydryl groups associated with the binding of GTP and tRNA. Crosshatched areas represent the segments whose amino acid sequences are being compared in fig.2.

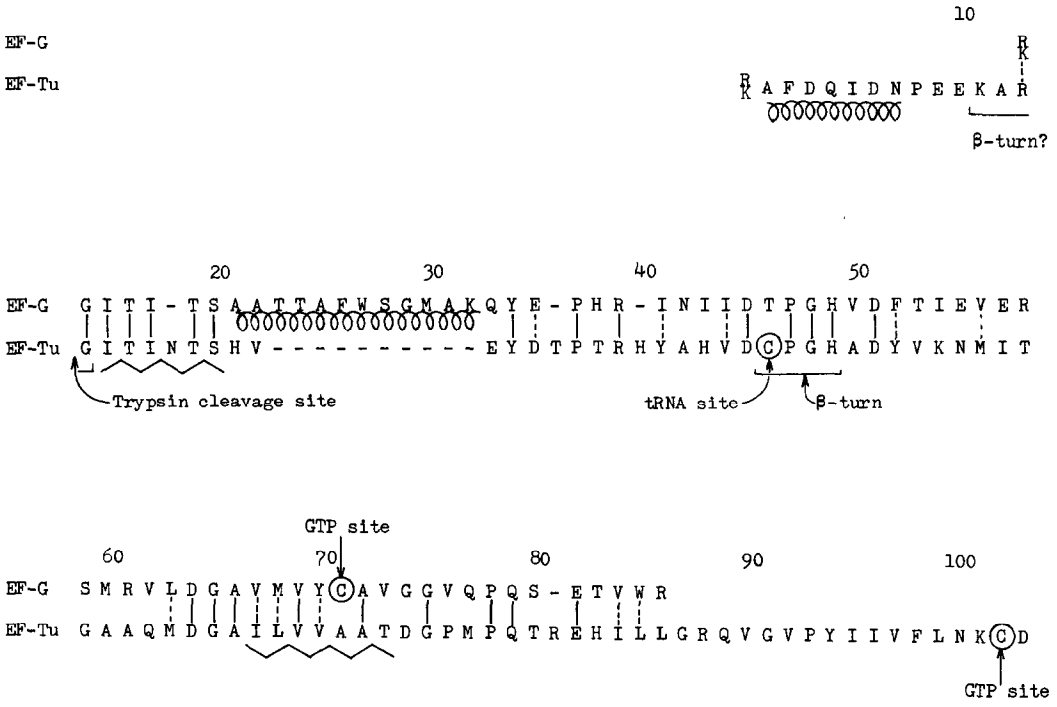

Fig.2. Comparison of amino acid sequences in EF-Tu and EF-G. Solid vertical lines indicate identical residues and broken vertical lines conservative replacements. Zigzag lines are predicted β-sheet regions common to both proteins; loops indicate α-helical regions in EF-G or EF-Tu. Selective cleavage by trypsin occurs at Arg_{13}–Gly_{14} in both proteins. The sequence data are from references 7, 9 and 11. Residues 1–13 were previously unpublished.

overall structure of this region of the two proteins is similar and that it may constitute part of the GTP binding site. On the other hand, there must be appreciable differences in structure between the two proteins, since antibodies to EF-G and EF-Tu do not cross react [12]. The fact that the two cysteines (residue 71 in EF-G and 102 in EF-Tu) reported [8,13,14] to be at the GTP sites are not in homologous regions is not necessarily a problem, because they may simply be in different areas of the GTP binding pockets. There is not yet any evidence that sulfhydryl groups participate in GTP binding, only that they are at or near the binding sites.

Another interesting structural feature is seen in the sequence at positions 13 and 14, Arg–Gly in EF-Tu and Arg– or Lys–Gly in EF-G [13]. In both proteins the Arg– or Lys–Gly bond is particularly susceptible to trypsin cleavage [9,15,16]. Moreover, actin, once suggested [17] to be homologous with EF-Tu, also has a sequence Arg–Gly–Ile–, which is selectively cleaved by trypsin. Recently Geisow [18] has compared amino acid sequences of a number of prohormones that are processed by proteolytic cleavage of an Arg-X bond. A characteristic of these cleavage points is that they lie in unstructured regions of the protein, usually between regions of α-helix or β-structure, or in β-turns. Similarly the trypsin suceptible Arg–Gly bond in EF-Tu appears to be in a β-turn between an α-helical and a β-structure region (fig.2) and is thus in a particularly exposed position on the surface of the protein.

Both EF-Tu and EF-G are involved in the processing of aminoacyl or peptidyl tRNA during protein biosynthesis and have GTPase activity directly or indirectly mediated by the ribosomal proteins L7 and L12 [3]. In view of these similarities it is perhaps not surprising that they have similar structural features. The simplest explanation is that EF-Tu and EF-G arose from a common ancestor by a process of gene duplication. Horowitz [19] has suggested that operons evolved from an ancestral gene by a process of gene duplications. Our data would support this idea, since EF-Tu and EF-G are adjacent to one another on the λ*fus* 2 operon [4].

EF-Tu and EF-G appear to be members of a new protein superfamily [20], the first observed among the participants in protein biosynthesis, and it will be of interest to see if there are other members. A likely candidate is IF-2, which binds formylmethionyl tRNA, has L7/L12 dependent GTPase activity and also, like EF-G and EF-Tu, is inhibited by thiostreptone [21].

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