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# Does a Bacterial Elongation Factor Share a Common Evolutionary Ancestor With Actin?

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Protein synthesis elongation factor Tu from E. coli shares several physical, chemical, and functional properties with actin-like proteins. Limited tryptic degradation indicates that the two polypeptides have a similar molecular architecture. These observations suggest that they could have evolved from a common ancestor, although more information will be necessary to prove or disprove this hypothesis. A partial sequence, comprising 22 aminoacid residues from the aminoterminal end of the large tryptic fragment of elongation factor Tu is presented.

Key words: elongation factor Tu, actin-like protein, limited proteolysis

## INTRODUCTION

Recently, we have described a major polypeptide from E. coli that appears to be associated, in part, with the bacterial membrane (1). With its mass of 42,000–44,000 daltons and approximately 70,000 molecules per cell, it constitutes about 5% of the total cell protein. Before we discovered its identity with elongation factor Tu (2), we suggested on the basis of several criteria (see below) that it may have a structural role at the membrane, and that its characteristics were reminiscent of actin-like proteins in nonmuscle cells (1). The recognition of its function as the factor (EF-Tu) that transfers aminoacyl tRNA to ribosomes made this hypothesis more intriguing since analogies between contractile processes and ribosomal translocation have been suggested on purely conceptual grounds (3, 4). Of course, it also opened the possibility for an alternative explanation of the membrane localization of EF-Tu: Not only could this protein provide a link of protein synthesis to the membrane (5), but it might also allow a complex coordination of macromolecular synthesis in bacterial cells. Although such an explanation may turn out to be the more significant one, we wish to address the first proposal here, since the question of whether actin-like proteins are characteristic for eucaryotic cells, or whether related proteins exist in procaryotic cells (6), has recently drawn increased interest. In the following discussion, we examine the extent and the limitations of the available evidence concerning a possible evolutionary relationship between the two proteins.

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## COMPARISON OF THE PROPERTIES OF ELONGATION FACTOR Tu AND ACTIN

The following characteristics first suggested to us that the two proteins might be related:

1. Both polypeptides appear to be located, in part, at the inner surface of the plasma membrane (1, 7), and both occur as major components in E. coli (1) and in nonmuscle cells (8), respectively.

2. Both proteins are quantitatively precipitated under certain conditions by an alkaloid, vinblastine, and are partially precipitated by calcium ions (1, 10). In eucaryotic cells, these latter characteristics have been postulated to be specific for "proteins derived from structure" (10).

3. Adhesiveness to surfaces, and a tendency to aggregate are characteristic properties of the elongation factor as well as of actin (1, 9).

A comparison of the physical and chemical properties of the two proteins shows the following similarities. Both polypeptides have masses of 42,000–44,000 daltons (1, 11, 12). Their amino terminal ends are blocked (2, 12). Both are acidic with isoelectric points of 5.3 and 4.7, respectively (1, 13). The hydrophobicity coefficient (14) is 42% in both cases (Ref. 1, and calculated from the amino acid composition given in Ref. 12). On inspection, their amino acid compositions (11, 12) are quite similar, and the calculation of their difference indices (15) confirms this. On a scale from 0 to 100 for identical to entirely different amino acid compositions, we have calculated a value of 11.4 for the two proteins. This compares to figures of 14 for human and mouse $\beta_2$ microglobulins (16), or to 13.9 between the $\alpha$ and $\beta$ chains of human hemoglobin (15). A prediction of the secondary structures (17) of EF-Tu and actin on the basis of their respective circular dichroism spectra (18, and quoted in 19) suggests that a major fraction of both proteins exists in a so-called random configuration. The values we calculated for $\alpha$-helix, $\beta$-sheet, and "random configuration" are 2%, 12%, and 86% for EF-Tu, and 13%, 16%, and 71% for actin. Functionally, the similarities of the two proteins consist in their ability to bind nucleotides and to stimulate a nucleoside triphosphatase activity of an associated protein complex, and in their dependence on divalent cations (13, 20). Also, both proteins have the inherent capacity to hydrolyze nucleoside triphosphates on their own under specific conditions (13, 21).

Equivocal results were obtained with antibodies to either EF-Tu or to actin. Immunoglobulin specific to the elongation factor released from E. coli by osmotic shock (2) reproducibly yielded a precipitation line in unidimensional immunodiffusion tests with actin as the antigen, and control experiments, performed in parallel with antibodies purified from preimmune serum, did not exhibit noticeable precipitation. However, we failed to reproduce this result with antibodies to EF-Tu purified according to the conventional procedure (11). The reverse experiments with antibodies specific to carboxymethylated actin (our unpublished procedure), or to actin, isolated from fibroblasts (3T3 SV101, the kind gift of Dr. R. Bloch) and prepared according to Lazarides and Weber (22), or to the protein from Physarum (kindly provided by Dr. B. Jokusch), also yielded negative results. In view of the notoriously poor antigenicity of both actin (22) and EF-Tu (23), this finding may not be surprising. However, owing to the tendency of actin to precipitate nonspecifically, the positive result mentioned needs further corroboration.

The differences that we have found between the two proteins may be summarized as follows. Elongation factor Tu does not contain methylhistidine (1, 11). Peptide maps, obtained as described (2) after tryptic hydrolysis or chemical cleavage

at methionyl residues, do not show striking similarities (result not shown). Sucrose gradient centrifugation (24) and electrophoresis under nondenaturing conditions (25) readily revealed the association of actin with deoxyribonuclease I, as has been described previously (9). Upon substitution of EF-Tu, however, we did not observe such an association. Functional differences are demonstrated by our inability to polymerize EF-Tu to a filamentous form, at least under the conditions identical to those we have used in an attempt to polymerize an actin fragment (Ref. 19; see also below). Furthermore, the factor fails to stimulate myosin ATPase activity, as assayed according to Spudich (26). We have also tested whether EF-Tu can copolymerize with actin. After addition of G-actin to an extract from $^{14}$C-amino acid labeled cells containing the elongation factor (1), conditions leading to the polymerization of actin (19) were induced. Gel electrophoretic analysis of the washed F-actin pellets, followed by staining and autoradiography (27), showed no perceptible copolymerization of EF-Tu with actin. Conversely, when actin (or a fragment thereof; see below) was added to a phenylalanine polymerization assay (28) instead of EF-Tu, it did not stimulate activity, nor did it inhibit that of EF-Tu when both proteins were added simultaneously. According to the stringent criteria that have been suggested to identify a protein as actin-like (29), a close relatedness of EF-Tu with actin is ruled out. It should be stressed, however, that we are not suggesting that EF-Tu is an actin-like protein, but rather that we wish to examine whether the two proteins may have a common ancestor.

## STRUCTURAL ORGANIZATION OF THE TWO PROTEINS: A DIFFERENT APPROACH

Recently, it has been recognized that multifunctional proteins, a class to which both EF-Tu and actin belong, often consist of several autonomous structural regions that may be separated by limited proteolysis (30, 31). Our initial study on the membrane association of EF-Tu (1) had shown that trypsin degraded this polypeptide to a relatively protease-resistant intermediate. We have therefore investigated this phenomenon further, and have conducted similar studies on actin with the rationale that the submolecular architecture of the two proteins may be a better indicator of relatedness than specialized functions. Using proteolysis of native molecules, these studies (18, 19) have revealed that both EF-Tu and actin exhibit an apparently similar architecture. Each molecule is characterized by a large, relatively protease-resistant carboxyterminal fragment (34,000–37,000 daltons) that binds nucleotides and contains the information necessary for its proper folding to an active structure from a random coil configuration. The aminoterminal segments of both proteins (approximately 7,000 daltons) are more labile after the initial proteolytic event. The two structural regions are connected by an exposed region of the polypeptide chain, which in either protein is hydrolysed rapidly when trypsin is added to native molecules (18, 19). It is noteworthy that the large fragment of EF-Tu is apparently able to perform all the functions it exerts in protein synthesis (18), whereas the protease-resistant core of actin cannot polymerize or stimulate myosin ATPase activity, in spite of the fact that it retains its full ATP binding capacity (19).

Limited proteolysis of native molecules has been observed in other proteins also (32). It is noteworthy, however, that two proteins that share a number of common properties also exhibit an apparently similar molecular architecture, as detected by this procedure. If this similarity reflects relatedness, it might be assumed that the aminoterminal ends of the two large tryptic fragments may provide a meaningful basis for

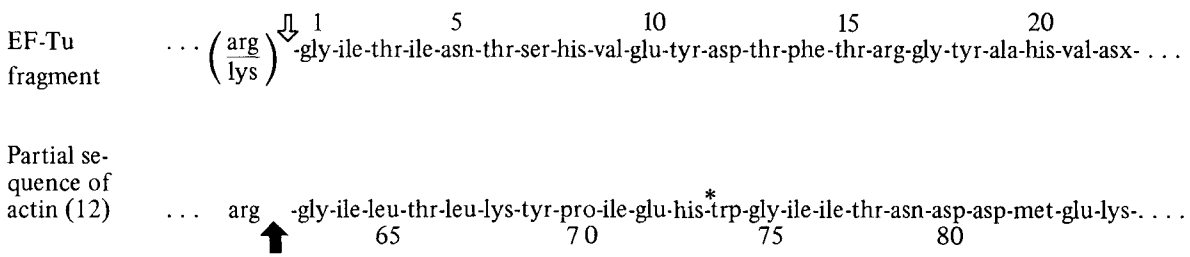


Fig. 1. A comparison of the aminoterminal sequences of the large fragments of EF-Tu and of actin (12). The open arrow indicates the tryptic cleavage site in EF-Tu (18), the closed arrow the primary cleavage site in actin (19). The asterisk at $his^{73}$ indicates that this amino acid is the $\tau$-methyl derivative of histidine. The aminoterminal sequence determination of the large EF-Tu fragment, prepared as described previously (18) was performed by automated analysis on a Beckman 890 B Sequencer, equipped with an undercut cup and nitrogen flush. The protein (200 nmoles) was degraded using the conventional Quadrol program (33). Thiazolinone derivatives, obtained after each degradation cycle, were converted to the phenylthiohydantoin (PTH) derivatives by incubation in 1 N HCl at 80° for 10 min. PTH amino acids were extracted twice with ethylacetate and dried under nitrogen. Identification of PTH amino acids was by gas chromatography (34) and by amino acid analysis after hydrolysis to the free amino acids with 65% (w/v) HI for 20 hr at 125° (35). In some cases, PTH amino acids were also assayed by thin layer chromatography (36). The average repetitive yield ranged between 92% and 93% in two consecutive sequence determinations.

sequence comparison. We have determined the first 22 aminoterminal residues of the large EF-Tu fragment. Figure 1 shows the alignment with the corresponding sequence of actin (12) localized as described previously (19). Although the sequences at and immediately following the cleavage sites are quite similar, the homology is limited to a small region, and the significance of this comparison is therefore questionable.

## CONCLUSIONS

The currently available information concerning a possible relationship between EF-Tu and actin does not allow an unequivocal answer to the question raised. The supporting evidence that we have presented is clearly circumstantial. The very large minimal evolutionary distance that separates the two proteins (37, 38) suggests that indeed only very limited homologies might be expected. This pertains not only to sequence homologies, but also extends to immunological cross-reactivity (15). Although we have obtained indications that such cross-reactivity may exist, the result presented remains to be confirmed, and its significance to be determined by more sensitive immunochemical methods. The negative evidence we have given is not conclusive, either. Differences in peptide maps may arise from much smaller differences in sequence (see Table 1 in Ref. 37) than those anticipated in the present case. The failure of EF-Tu to polymerize may either be due to the conditions used or could be the consequence of a strongly divergent evolution of the aminoterminal fragment, which we have shown to be necessary for polymerization of actin, but which apparently is not required for the activity of EF-Tu (18, 19).

To us, the comparison of the molecular architecture is more interesting. Very elegant studies of the tertiary structure of proteins at a high level of resolution have recently shown that specific protein domains may be preserved over large evolutionary distances (37, 39), even if the present day functions of the proteins are unrelated. High resolution analyses of the three-dimensional structures of many proteins cannot be expected in the near future. Therefore, the coarse approach to which we have resorted to examine submolecular organization may prove useful in other cases as well, provided of course that relatedness can be ultimately established by independent means.

As indicated in the introduction, a recent report has claimed the existence of an actin-like protein in E. coli (6). Based on a molecular weight of 45,000 and its abundance in the cell, it is likely to be identical with the elongation factor considered here. Under defined conditions, this protein appears to aggregate and to interact with myosin. We have repeated the experiments presented in that account by attempting to coprecipitate EF-Tu either in homogeneous form (11), or in unfractionated cell extracts (6), with an excess of pure rabbit muscle myosin. We found only about 5% of the total EF-Tu in either case associated with the myosin. Due to the adhesiveness of EF-Tu, we feel that caution must be exerted in drawing conclusions from this association.

In view of the significance that an unequivocal answer to the question raised in this discussion may have, we believe that the possibility of an evolutionary relationship between EF-Tu and actin merits further attention. However, it may well be that only the elucidation of the tertiary structures of the two proteins can provide a definitive answer.

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