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Original Research Article

Role of Elongation Factor EF-Tu in Bacterial Cytoskeletons - Mini Review and Update

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The existence of bacterial cytoskeletons – besides a structural cellular element named FtsZ involved in cell division – was not known. It was even proposed that possession of a cytoskeleton is one of the major structural features of higher organisms. The situation changed when actin-related bacterial proteins and a variety of other structural elements with cytoskeletal functions were discovered. Bacterial elongation factor EF-Tu was not classified as such an element. After all, it was known as a factor involved in bacterial translation and elongation. In the meantime, however, experimental data were collected which show that EF-Tu has a double function, i.e. not only in translation and elongation, but also as an intrinsic component of bacterial cytoskeletons in general.

Keywords: Bacterial Cytoskeletons, Elongation Factor EF-Tu, EF-Tu with Double Function, Aspects of Evolution, Recombinant Bacterial Strains, Induced Bacterial Lysis, New Class of Antibacterial Agents.

INTRODUCTION

The prokaryotic three-domain protein elongation factor EF-Tu (Song et al.,1999) (Fig. 2) is known as an intrinsic component in the process of translation and elongation. It is present in the bacterial cell in high copy number (around 100,000 or more copies per cell), but only about 25 % of these copies are involved in translation/elongation (Furano,1975, Jacobson and Rosenbusch,1976). Formation, *in vitro*, of fibrils composed of EF-Tu was observed (Jacobson and Rosenbusch,1976, Beck et al.,1978, Cremers et al.,1981). This observation supported the notion that EF-Tu does not only have a function in translation/elongation, but may rather be involved in other cell functions, e.g. that of intracellular transport.

The existence of bacterial cytoskeletons – besides a structural element named FtsZ that is involved in cell division (Bi and Lutgenhaus,1991, Erickson et al., 1996) - was not known. Until then, it was even proposed that possession of a cytoskeleton is one of the major structural features of cells of higher organisms (mentioned by Hempel and Schwienhorst, 2005). For many years the idea of a bacterial cytoskeleton network in general and of a double function of EF-Tu remained ignored.

RESULTS AND DISCUSSION

In 1987, a surprising observation was published (Antranikian et al.,1987) that indicated the presence of a kind of cytoskeleton in bacteria besides the FtsZ ring. This observation was backed in 1998 by application of immunoelectron microscopic techniques (Mayer et al.,1998). The data supported the view that prokaryotes may contain cytoskeletal elements that are responsible for maintenance of cell stability and shape.
Later on, various research groups collected a multitude of data on prokaryotic cytoskeletons composed of various kinds of proteins, especially of actin-related components (Mayer, 2002, Hegermann et al., 2002, Carballido-Lopez and Errington, 2003, Löwe et al., 2004, Madkour and Mayer, 2007, Graumann, 2007).

In addition, papers were published speculating on evolutionary aspects of the cytoskeleton of higher organisms and their relationships with bacterial cytoskeletal components (Van den Ent et al.,2001a, Van den Ent et al., 2001b). In papers published in 2002 (Mayer 2002), in 2006 (Mayer, 2006) and in a status report and hypothesis paper (Mayer, 2003) data were summarized that had been, in the meantime, collected by experiments performed to show that EF-Tu is one of the important components of bacterial cytoskeletons in general. It was suggested that an EF-Tu-based bacterial cytoskeleton might be the primordial kind of cytoskeleton. This

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would mean that other kinds of bacterial cytoskeletons, e.g. actin-related ones, would have developed later.

Hence, the early notion on a double function of EF-Tu was "rediscovered". Since then, very interesting relevant data were communicated: in 2010 a paper with the title "Bacterial translation and elongation factor EF-Tu interacts and colocalizes with actin-like MreB protein" was published (Defeu Souto et al., 2010), followed, in 2015, by an article with the title "Translation and elongation factor EF-Tu modulates filament formation of actin-like MreB protein *in vitro*" (Defeu Souto et al., 2015). In summary, now EF-Tu is considered to be a major ubiquitous component of bacterial cytoskeletons. It was even suggested that EF-Tu filaments may serve as tracks for MreB filaments (Defeu Souto et al., 2010). From this point it might not be a very long way to confirm that an EF-Tu-based bacterial cytoskeleton was the primordial cytoskeleton as suggested earlier (Mayer,2002).

REGARDING EVOLUTION

One could speculate (and perhaps later confirm) that filament formation for preservation of bacterial cell stability and shape might not have been the very first property of EF-Tu; an even more important function of EF-Tu might have been its involvement in the translation system, i.e. in a process that can be assumed to be very "primordial". After all, any cell shape-stabilizing cytoskeletal network needs proteins. Filament formation by EF-Tu might then be a property acquired later in evolution, followed by the evolution of other kinds of bacterial cytoskeletons.

REMARKS

In a paper published in 2007 (Madkour and Mayer, 2007) on the existence of bacterial cytoskeletons it was shown, in *Escherichia coli* cells, that bacterial ribosomes formed rows along helically arranged filaments located close to the inner surface of the cytoplasmic membrane. Assuming that these filaments were composed of EF-Tu, one could speculate that translation - the classical ribosomal function into which EF-Tu is involved- takes place at ribosomes transiently complexed with EF-Tu "immobilized" in EF-Tu filaments. Such a view, if shown to be true, would be a convincing illustration of the double function of a bacterial protein.
Aspects of application of the discoveries regarding EF-Tu: two patents were awarded.

Patent WO 2004005 506 A2: Induced lysis of bacteria
Patent WO 2002087 554 A2: Design of a new class of antibiotics *("Nanocillins")* with EF-Tu as the target.

Candidates for *Nanocillins* could be small peptides, designed along the known amino acid sequences in the cleft and the loop (s. above), i.e. with a sequence/motif that allows competition for binding with the respective binding sites in native full-size EF-Tu proteins (cleft in domain 2, loop exposed on domain 3 (s. Fig.2). Their design could even be strain-specific, under consideration of the amino acid motifs in the cleft and in the loop of given bacterial strains. However, uptake of these small peptides, with a function as antibacterial agents, into the cytoplasm of the bacterial cell might cause problems.

Therefore, any other compound (small molecule) with such a property would be a realistic candidate as well. A search for such a compound could be performed as follows: compounds known to bind to proteins, taken from a library of compounds existing anyway, could be investigated by measuring, with physical techniques, their potential to prevent complex formation (i.e. formation of filaments) out of free individual full-size EF-Tu proteins in a solution known to favour filament formation in the absence of any kind of inhibitor. Optimization could be achieved by variation of the numerical ratio of the components (EF-Tu protein and competitive inhibitor of filament formation) in the solution. It may be expected that uptake of such a kind of small compound into the bacterial cell should not cause severe problems. It may be mentioned that, in principle and in general, a selection of other components of bacterial cytoskeletons as targets for additional examples of the new class of antibiotics *("Nanocillins")* may support the struggle against resistance.

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Figs. 1 to 8 illustrate structural aspects of bacterial cells (*Escherichia coli*) and EF-Tu observed and used as a basis for the patents mentioned above

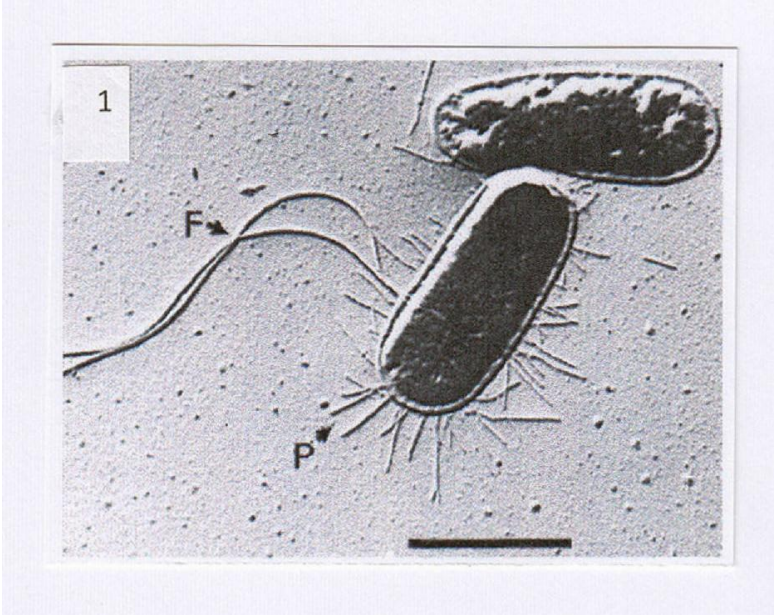

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Fig. 1: *Escherichia coli* cells harvested from a standard culture. Cells were flattened by the preparation procedure (metal shadowing) for transmission electron microscopy; otherwise the cells appear to be undamaged (F, flagella; P, pili); compare with *Escherichia coli* cells after induced lysis (Figs.8a und 8b). Bar: 1 micrometer. Original micrograph by Frank Mayer

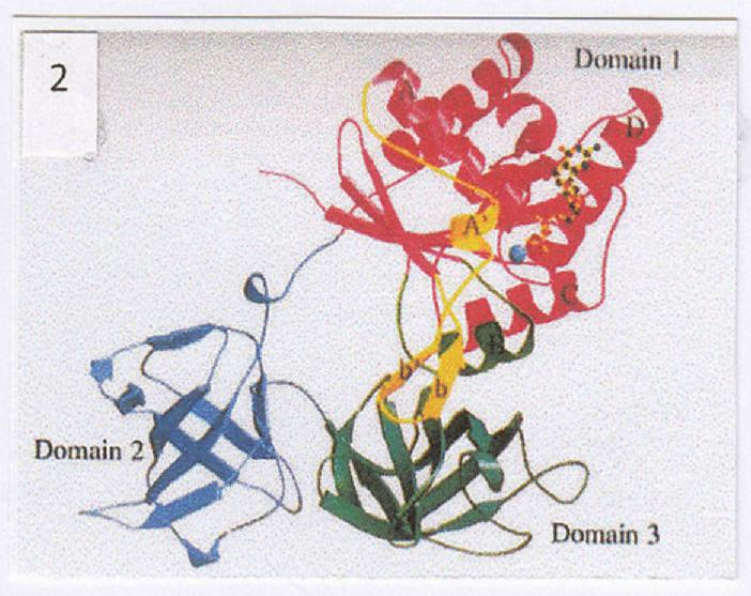

Fig. 2: Ribbon plot of full-size EF-Tu in the GDP state. Note: the protein exhibits three domains. Domain 1 is, together with parts of domain 2, involved in translation. Domain 3 has an exposed loop extending from its surface; domain 2 has a cleft. (From Song et al.,1999)

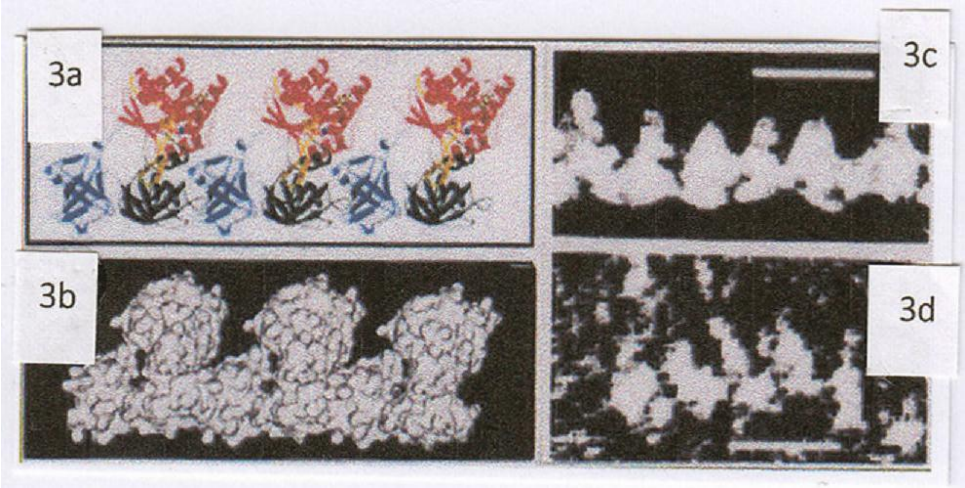

Fig. 3:

a. Model for the formation of a filament (by polymerization) composed of EF-Tu protein molecules, taking advantage of the fact (confirmed by computer simulation) that the loop of domain 3 fits into the cleft of domain 2 of a neighboring full-size EF-Tu protein. (From Madkour and Mayer, 2007)

b. Modelling of the situation depicted in Fig.3a. Modelling done by Andreas Schwienhorst

c. Stretch of a negatively stained filament isolated from *Thermoanaerobacterium* spec. printed with enhanced contrast. (From Mayer, 2006)

d. Stretch of a filament, generated from isolated monomeric *E. coli* EF-Tu. Negative staining and contrast enhancement. (From Mayer, 2006)

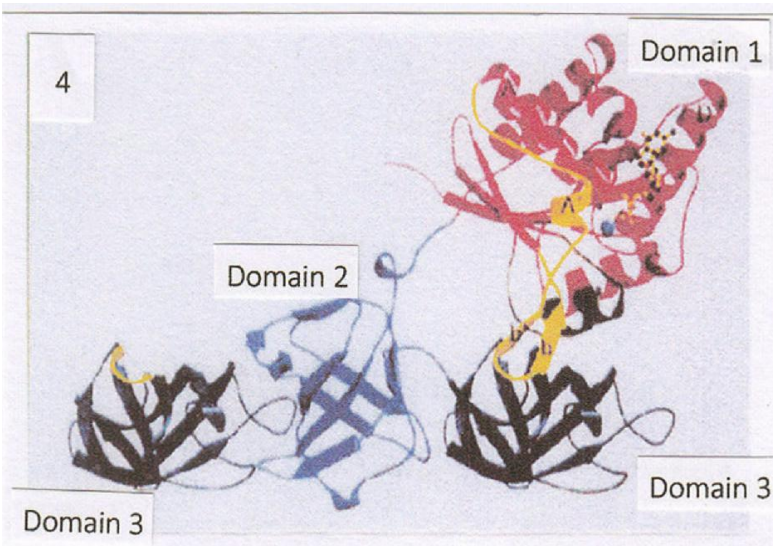

Fig. 4: Prevention of filament formation by insertion of a kind of EF-Tu that is truncated (this molecule was designed and synthesized in *Escherichia coli* by Schwienhorst and Hempel using recombinant techniques). It consists solely of domain 3 of the full-size EF-Tu protein. As soon as such a molecule is bound to a full-size EF-Tu, filament elongation in one direction is prevented because there is no possibility for the binding of the domain 3 of a neighboring full-size EF-Tu. The respective site on domain 2 of the full-size EF-Tu is already occupied by the truncated EF-Tu. If this situation is created within an *Escherichia coli* cell by induced synthesis of truncated EF-Tu in a certain numerical ratio compared to full-size EF-Tu molecules, the consequence is a drastic destabilization of the cytoskeleton responsible for maintenance of cell shape and stability. (From Mayer, 2006). Compare with Fig.8a and 8b)

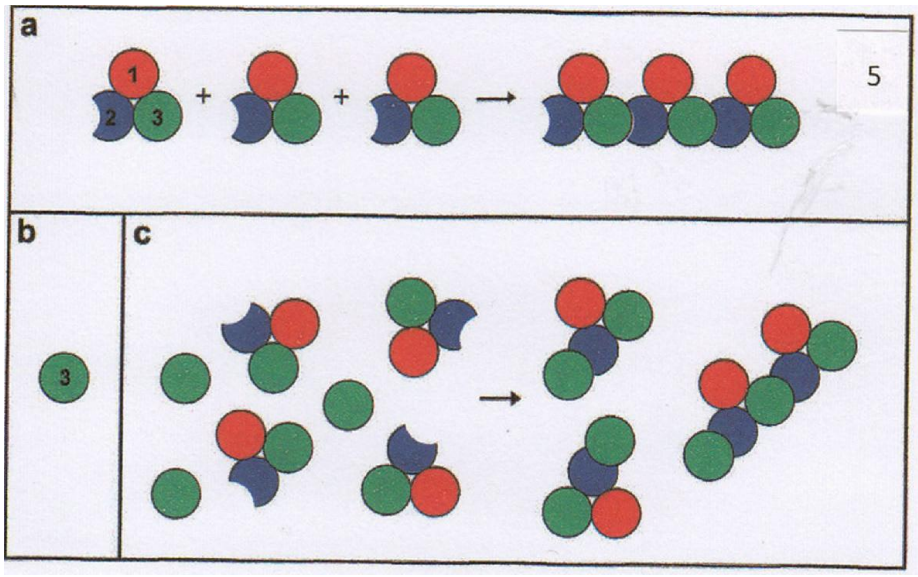

Fig. 5: Sketch illustrating prevention of filament formation by insertion of a truncated EF-Tu protein consisting solely of domain 3 (shown in green) (compare with Fig. 4). (From Mayer, 2006)

a. Situation without addition of the truncated EF-Tu protein: filament formation
b. Truncated EF-Tu protein consisting solely of domain 3
c. Situation after addition of truncated EF-Tu protein copies to a sample containing intact EF-Tu proteins: prevention of filament formation

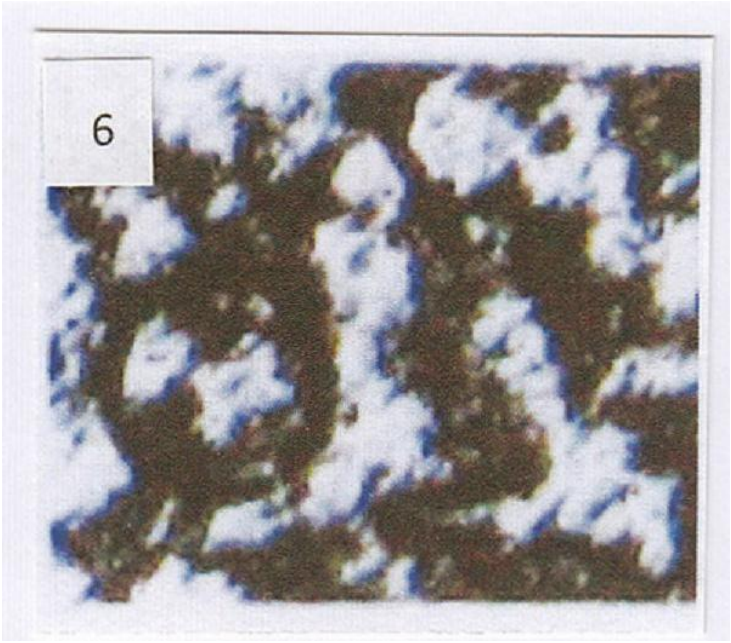

Fig. 6: Electron micrograph (negative staining, enhanced contrast) of a sample prepared, *in vitro*, according to Fig. 5 c. Filament formation is prevented due to the presence of truncated EF-Tu.(From Mayer, 2006)

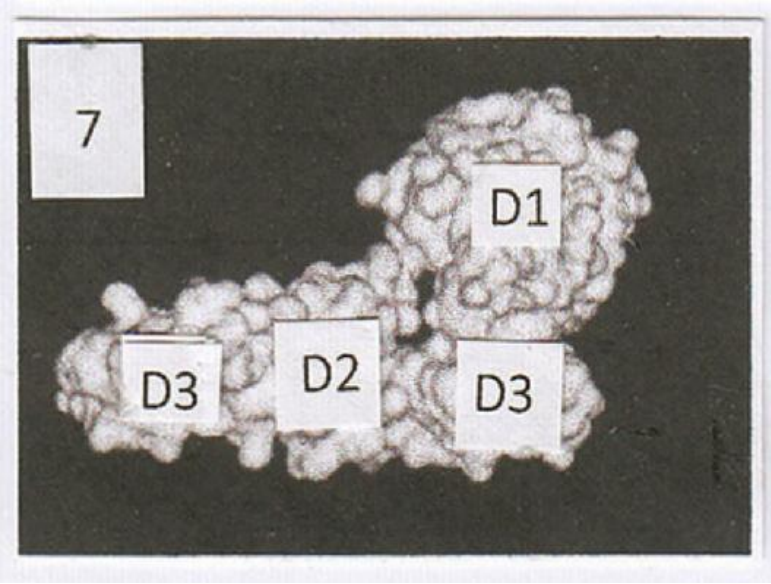

Fig. 7: Modelling of the situation depicted in Fig. 4. A complex with a shape very similar to shapes seen in Fig. 6 is depicted. Modelling was done by Andreas Schwienhorst. (From Mayer, 2006)

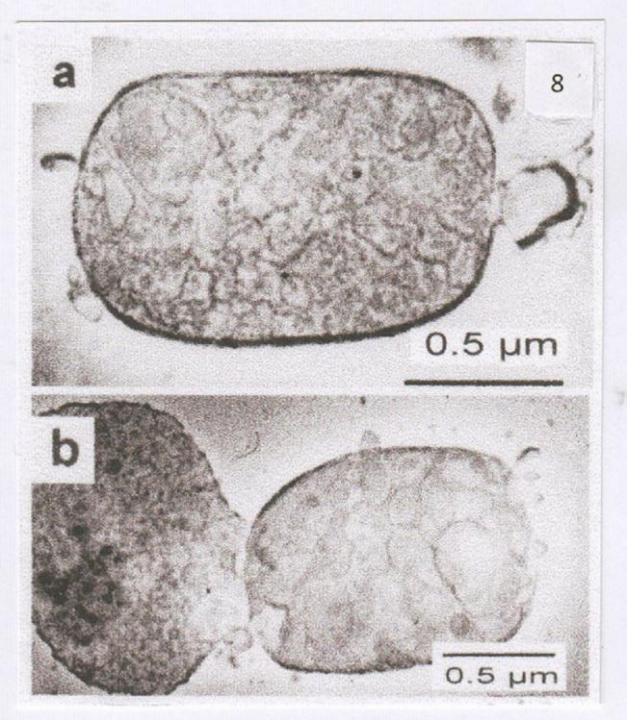

Fig. 8 *Escherichia coli* cells after onset of induced lysis, i.e. after the onset of induced synthesis of truncated EF-Tu proteins. This approach results, as depicted in Figs. 4 to 7, in destabilization of EF-Tu filaments and, hence, of cell stability and shape and complete loss of cell integrity. Induced lysis allows harvesting of products, synthesized within the cells by application of recombinant techniques, without any physical or chemical stress. This feature may be important, e.g. for high yields of produced intact covalently closed double-stranded nucleic acids for gene therapy or of other products sensitive to shear forces or chemical treatment used for cell lysis. (From Mayer, 2006)