Immunocytochemical localization of the elongation factor Tu in *E. coli* cells

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The localization of the elongation factor Tu (EF-Tu) in ultrathin cryosections of *E. coli* cells was determined with the electron microscope using a highly specific immunological labelling technique. EF-Tu is distributed almost homogeneously throughout the cytoplasm. Although it has often been suggested that EF-Tu could be part of a putative prokaryotic cytoskeleton, we did not find any evidence for supramolecular assemblies, such as fibres or filaments, containing a large amount of EF-Tu. EF-Tu was not observed in association with the outer cell membrane and periplasmic space. A topological relationship with the inner membrane is not apparent in our micrographs. In cells in which the EF-Tu level is raised significantly, the protein piles up in discrete cell regions.

Elongation factor Tu E. coli *Immunoelectron microscopy* *Ultracryotomy* *Colloidal gold labelling*

1. INTRODUCTION

One of the most abundant proteins in *E. coli* cells is EF-Tu [1,2]. Beside its well documented role in protein biosynthesis [3–5] and bacteriophage RNA replication [6,7] a number of other functions have been ascribed to this protein [8–13]. Similarities between some properties of EF-Tu and those of the eukaryotic protein actin have led to speculations about a structural role of the elongation factor as part of a presumed prokaryotic cytoskeleton. These properties are the ability to form bundles of EF-Tu fibres [14,15], cylinders [16], a reported peripheral association with the plasma membrane of *E. coli* [17], quantitative precipitation with vinblastine and calcium ions [18], and particularly its abundance in the bacterial cell [1,2,17].

To shed more light on the putative structural role of EF-Tu and its topological relationship to the inner membrane we have investigated the localization of this protein in *E. coli* cells. We have applied a technique which enabled us to label EF-Tu in situ [19,20]. Ultrathin (50–100 nm) cryosections of *E. coli* cells were treated with antibodies raised against EF-Tu. The complexes thus formed were visualized with the electron microscope after labelling with protein A bearing colloidal gold particles.

Abbreviation: EF-Tu, elongation factor Tu

2. EXPERIMENTAL

Wild-type *E. coli* strain LBE 1001, in which 5% of the total cellular protein is EF-Tu [2], and strain LBE 12020 AsBo,pCI,pGp81, were grown in LC medium at 37°C. The experimental conditions for culture of the latter strain and elevation of the intracellular EF-Tu concentration are described in detail in [13]. Cells were harvested by centrifugation, slightly fixed with 2% paraformaldehyde/0.5% glutaraldehyde in 0.1 M phosphate buffer, pH 7.2 (PB) and washed 3 times with 2% paraformaldehyde/1 M sucrose in PB. Rabbit anti-EF-Tu was prepared as in [21] and purified

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with affinity chromatography ([22] with minor modifications), using EF-Tu coupled to a CNBr-activated Sepharose 4B carrier. Fixed cells were suspended in 10% gelatin in PB at 37°C and pelleted. The pellet was squeezed to an about 0.5 mm thick slab, allowed to solidify on ice and fixed for 30 min in the same paraformaldehyde/glutaraldehyde mixture. Fixed protoplasts [23] were embedded in 30% polyacrylamide (PAA) as in [19]. From this embedded, fixed material, cubic blocks of about 0.5 cm were prepared and, after infusion with 2.3 M sucrose they were rapidly frozen on specimen holders in liquid nitrogen. Ultrathin cryosections were then cut and transferred to carbon formvar coated copper grids [20]. Immunolabelling of the sections with anti-EF-Tu IgG (50 μg/ml) was done as in [24] using protein A colloidal gold complexes [25] as an indirect marker of the antigenic sites. Control sections were treated according to the same procedure, but anti-EF-Tu was replaced by non-specific rabbit IgG. Virtually no gold particles were observed in the control sections. After immunolabelling the sections were uranyl stained and embedded in 1.5% methyl cellulose [20]. Fig. 1–6 are from ultrathin cryosections of gelatin (1–5) and PAA embedded *E. coli* cells, which were immunostained against EF-Tu with 4–5-nm gold particles.

3. RESULTS

The ultrastructure of *E. coli* is visualized fairly well after the uranyl-methylcellulose treatment of the cryosection. The outer and inner membrane of the cell envelope, with the periplasmic space in between can be appreciated when the plane is perpendicular to the cell's surface (fig.1,2). The intracellular space is occupied by fine granular material except for a 'light' central area. The cytoplasmic material has a similar appearance in longitudinal (fig.1) and cross (fig.2) sections. Fibrillar structures are not observed.

The immunogold labelling reveals that in normal cells EF-Tu occurs homogenously distributed in the cytoplasmic material. Labelling of the light central areas is much less and mainly restricted to some floccules of cytoplasmic material occurring there. The cell envelope is not significantly labelled (fig. 1,2).

Although EF-Tu is a very abundant protein with intracellular concentrations varying from 5 to 10% of the total bacterial protein, far in excess over EF-

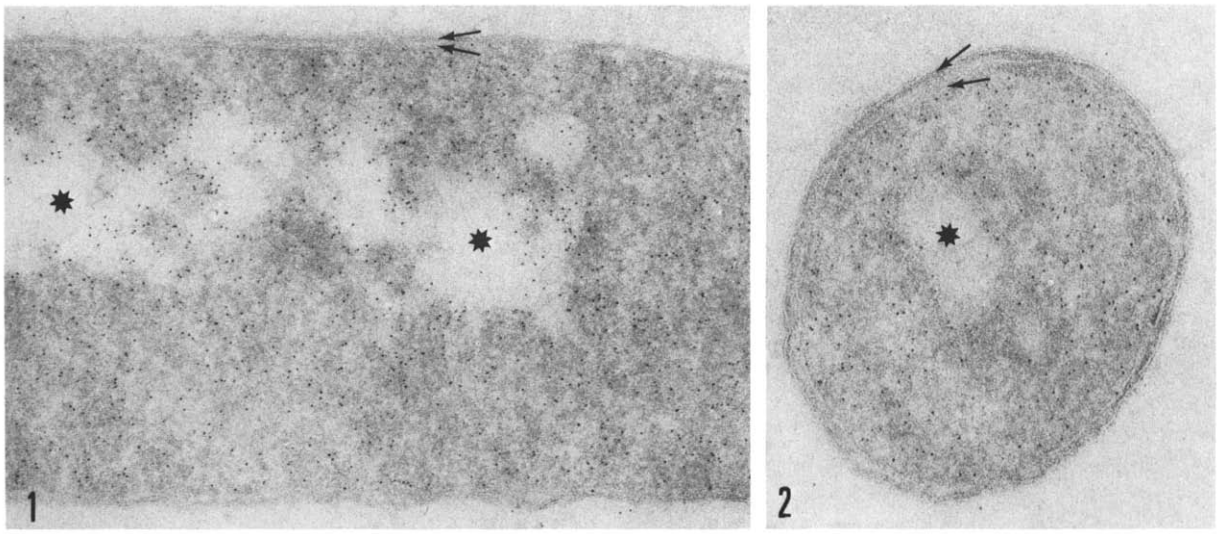

Fig. 1. *E. coli* LBE 1001 (wild type). Gold particles are spread over the cytoplasmic material. The light central area (asterisks) and the periplasmic space between the inner and outer membrane of the cell envelope (arrows) are not labelled. 60000 ×.

Fig. 2. Same as fig. 1, cross section. 60000 ×.

Ts and ribosomes [1,2], it probably is not present in the free form, but as a ternary complex with GTP and aminoacyl-tRNA. As such it displays little tendency to aggregate, as indicated by neutron scattering experiments, showing that the radius of gyration remains constant upon increasing the concentration of the ternary complex [26]. We therefore investigated the localization of EF-Tu in cells in which the EF-Tu level was raised significantly above the tRNA level. This was accomplished by transforming *E. coli* cells with a multicopy plasmid, harbouring tufA coding for EF-Tu. TufA was cloned on the plasmid pGp81 behind the powerful major leftward promoter P_L of phage λ. Transcription from P_L is repressed at low temperature by a temperature-sensitive λ repressor and activated by raising the temperature to 43°C [13].

Cells of strain LBE 12020 AsBo,pCI,pGp81, grown at 37°C, contain about twice as much EF-Tu as the wild type. In these cells the EF-Tu distribution observed is identical, although the labelling density is considerably higher (fig.3). Raising the temperature to 43°C induces dark granules in most cells (fig. 4). In sectins of gelatin-embedded cells, these granules are labelled for EF-Tu also (fig.5), with an intensity not very different from that of cytoplasmic labelling. However, the cytoplasmic material seems much looser, and immuno-reagents may penetrate deeper than in the dense granules, resulting in much more efficient labelling of EF-Tu. We therefore embedded protoplasts of these cells in PAA (protoplasts were used to enhance penetration of PAA constituents). In contrast to gelatin, PAA forms an intracellular meshwork [19], which permits uniform immunolabelling restricted to the whole, equating the labelling conditions for loose and dense material of the cytoplasm. In such cryosections of PAA embedded material we observe a considerably denser labelling of the granules than the cytoplasmic material (fig. 6). Counting of gold particles per μm^2 in 10 randomly chosen cells in a specimen revealed 4–5-times denser labelling of the granules. This suggests that EF-Tu is concentrated in these granules.

4. DISCUSSION

The data obtained here with a highly specific immunological labelling technique [19,20] have not yielded evidence for the occurrence in *E. coli* of EF-Tu in supramolecular assemblies of fibres and filaments reminiscent of a cytoskeleton. Although

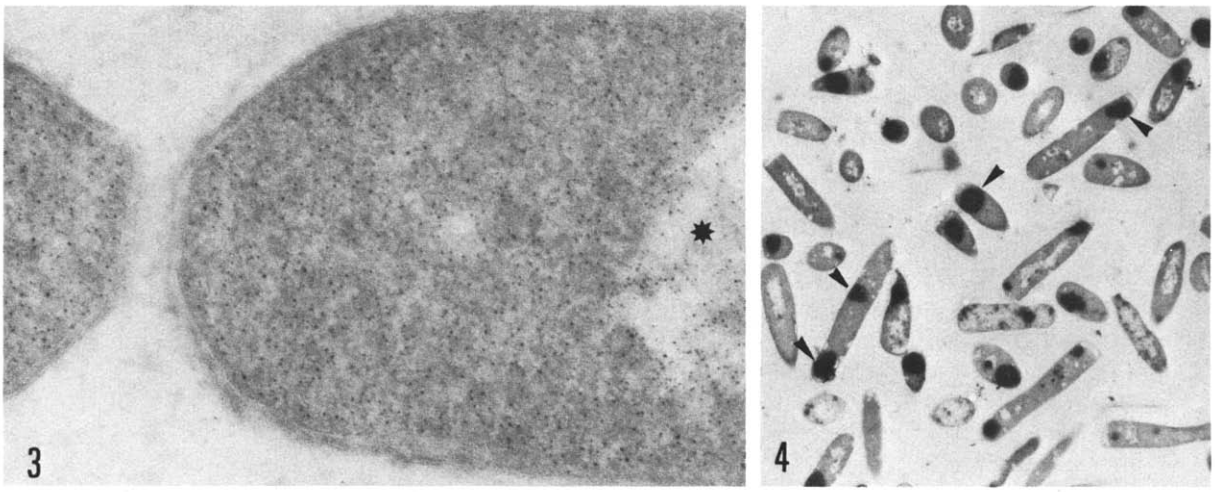

Fig. 3. Strain LBE 12020, AsBo,pCI,pGp81, cultured at 37°C. Gold particles are distributed as in the wild type, but the labelling is more dense. 60 000 ×.

Fig. 4. Strain LBE 12020, AsBo,pCI,pGp81, after heat activation. Dark granules are present in most cells (arrowheads). 3 000 ×.

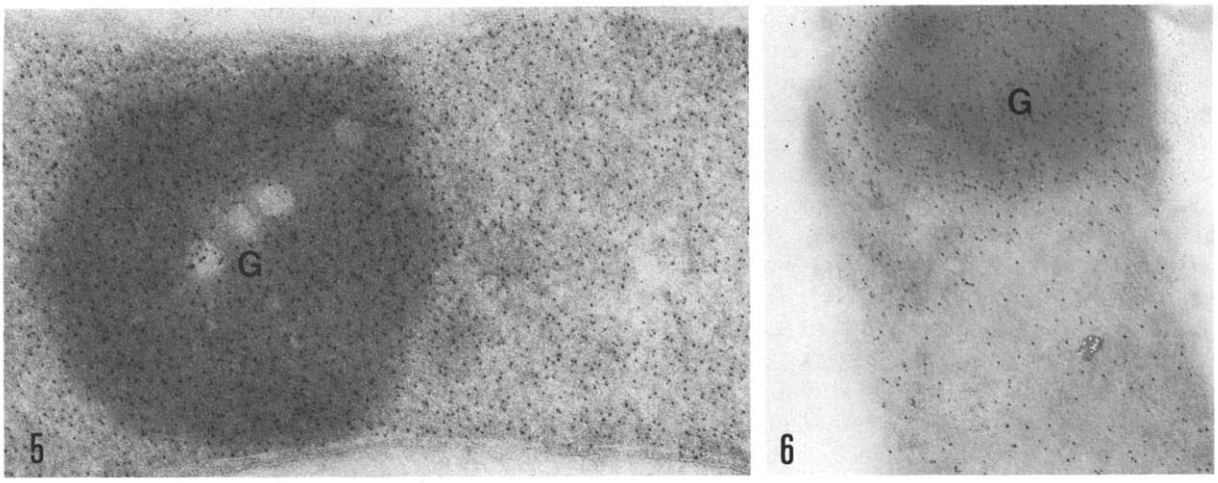

Fig. 5. Strain LBE 12020, AsBo,pCI,pGp81, after heat activation. In addition to the cytoplasmic material dark granules (G) are labelled. 60 000 ×.

Fig. 6. Strain LBE 12020, AsBo,pCI,pGp81, after heat activation, protoplasts. Uranyl-methylcellulose treatment was omitted. In this PAA-embedded specimen the granules (G) are more densely labelled than the rest of the cytoplasmic material. 50 000 ×.

the technique has been shown to be effective in revealing the eukaryotic cytoskeleton elements [27,28], the present data do not exclude EF-Tu being part of a more subtle nework, the detection of which is beyond the resolution power of the applied procedure. Our observations do not confirm the report by authors in [29] who also observed in cryosections EF-Tu localized in the cell envelope. Possibly they misinterpreted non-specific labelling of the envelope after using high concentrations of antiserum. In fact after using more dilute antiserum in their picture envelope-staining is not obvious. Our results clearly demonstrate that no significant amount of EF-Tu occurs in the cell envelope.

Our results neither confirm previous reports [17] that EF-Tu is peripherally associated with the inner membrane. Accumulation of gold particles in close proximity of the membrane is not observed. An indirect attachment of EF-Tu however via an intracellular network connected with the membrane remains possible.

Under normal growth conditions the intracellular EF-Tu level keeps pace with the tRNA level [1]. This suggests that it is present as a predominantly ternary complex with aminoacyl-tRNA and GTP, which seems to have less tendency to aggregate than has the EF-Tu · GDP complex [24]. Raising the EF-Tu level considerably (to 30% of all bacterial protein) above that of tRNA by transforming *E. coli* cells with a multicopy plasmid harbouring tufA behind the powerful P_L promoter of λ does not alter essentially the picture described above. Neither aggregation into fibres nor direct association with the plasma membrane becomes apparent. Noteworthy however is the accumulation under these conditions of EF-Tu in discrete regions, the dark granules. These granules are commonly observed when products pile up after gene cloning [30]. This is the first demonstration in situ that they indeed contain accumulated gene products. Cells from strain LBE 12020,AsBo,pCI,pGp81, cultivated at 37°C, which have an elevated intracellular EF-Tu concentration of 17%, do not show these regions. Apparently storage in these regions only occurs when the EF-Tu level exceeds a certain value. The underlying mechanism is unknown. A further characterization of these EF-Tu accumulations, which may have technological significance, is underway.

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