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Novel properties of bacterial elongation factor Tu

(polymerization complex/elongation factor Ts/filaments/DNase I binding)

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ABSTRACT **We have characterized novel properties of the bacterial protein synthesis elongation factor Tu which indicate that it may function as a structural protein. Under appropriate conditions, elongation factor Tu polymerizes to form filaments and, more often, bundles of filaments. It is also the predominant component of a complex of proteins from *Escherichia coli* that undergoes reversible polymerization in the presence of KCl and $MgCl_2$. In addition, purified elongation factor Tu binds tightly to DNase I in the presence of 10 mM $MgCl_2$. In crude extracts the factor shows no binding in the presence or absence of $MgCl_2$. These properties suggest that elongation factor Tu may have certain actin-like properties and that it has cellular functions other than its role in protein synthesis.**

It is well established that the elongation factor Tu (EF-Tu) is involved in bacterial protein synthesis and is a component of bacteriophage Qβ replicase (1, 2). It has also been proposed that EF-Tu is involved in the regulation of ribosomal RNA synthesis (3). According to the models proposed in each case, EF-Tu functions in the monomeric form and is, presumably, freely soluble inside the cell. It is therefore intriguing that purified EF-Tu aggregates readily *in vitro* and precipitates in the presence of calcium and vinblastine ions (4, 5), which is more characteristic of "proteins derived from structure" than of soluble proteins (6). Jacobson *et al.* (5) have pointed out that these properties are shared by actin.

Minkoff and Damadian (7) have isolated a complex of proteins from *Escherichia coli* by procedures similar to those used for isolating actin and have demonstrated that a component of the complex has the same molecular weight as actin and also that this component might be selectively removed from the complex by interaction with myosin. We have repeated the procedures of Minkoff and Damadian (7) and have shown that the actin-like component they described is identical to EF-Tu. We have also tested EF-Tu prepared by the usual procedure of Miller and Weissbach (8) for two other actin-like properties: the ability to form filaments *in vitro* and to interact with DNase I. Our results indicate that EF-Tu does form filaments and bundles of filaments under certain conditions. We were unable to demonstrate an affinity for DNase I under the conditions described for actin (9) but, with the addition of 10 mM $MgCl_2$, purified EF-Tu appears to bind DNase I.

MATERIALS AND METHODS

Strains and Media. *E. coli* K-12 strain HB101 (F^-,*ram* C1, *pro*$^-$,*gal*$_1^-$, *str*$_1^r$, rec$^-$r^-_br^-_m) was obtained from Ray White. Cells were grown in L broth (10) at 37°.

Proteins. For preparation of the polymerization complex, cells were grown to A_{585} = 1.0, harvested, washed twice with 0.5 mM Tris·HCl (pH 7.5), and disrupted by ultrasonic oscillation in six 10-sec bursts with a Branson sonifier. The sonicated preparation was polymerized and depolymerized by using the conditions described by Minkoff and Damadian (7) except that 0.1 mM dithiothreitol (DTT) was used instead of 2-mercaptoethanol and $MgCl_2$ was used instead of $MgSO_4$. EF-Tu was generously donated by David Miller, Herbert Weissbach, and Andrea Parmeggiani. EF-Tu was routinely stored at −80°, at 5 mg/ml, in 20 mM Tris·HCl, pH 7.5/2 mM $MgCl_2$/0.5 mM DTT/0.25 mM GDP/0.1 mM EDTA. EF-Tu antibody was a gift from Thomas Blumenthal. Protein concentrations were determined by the Hartree modification (11) of the method of Lowry *et al.* (12).

Polyacrylamide Gel Electrophoresis. Sodium dodecyl sulfate ($NaDodSO_4$)/polyacrylamide slab gels were prepared according to the method described by Laemmli (13). The running gel, consisting of a linear gradient of 7.5–15% acrylamide, was allowed to sit at least 16 hr with a few milliliters of gel buffer layered on top prior to pouring of the stacking gel. Gels were run for 6 hr at 175 V and then stained and destained as described by Beck and Park (14). Standard protein molecular weight markers, purchased from Sigma Chemical Company, were hemoglobin (15,500), carbonic anhydrase (29,000), ovalbumin (45,000), and bovine serum albumin (68,000).

DNase I-Sepharose Column Chromatography. Pancreatic DNase I (Worthington Biochemical, cat. no. 2007) conjugated to cyanogen bromide-activated Sepharose 4B (Pharmacia) was prepared by the method of Lazarides and Lindberg (9). Approximately 5 mg of DNase I was bound per g of Sepharose 4B. Samples were applied to a DNase-Sepharose column (3-ml bed volume) in 10 mM Tris·HCl, pH 7.9/10% glycerol/1 mM $CaCl_2$/0.1 mM DTT (buffer I). The column was subsequently washed with 2 volumes of buffer I, and eluted stepwise with 0.3 M guanidine HCl in buffer I and 3.0 M guanidine HCl in buffer I. Fractions from the absorbance peaks were pooled, dialyzed against distilled water at 4°, and lyophilized prior to application to $NaDodSO_4$/polyacrylamide gels.

Electron Microscopy. Ultrastructure of protein samples was examined with a Philips 300 electron microscope. Samples were applied to 200-mesh copper grids (Pelco) coated with Formvar and carbon, rinsed with 1 drop of 0.1% cytochrome *c*, and negatively stained with 1% uranyl acetate.

RESULTS

Characterization of EF-Tu as the Major Protein of the Polymerization Complex. We repeated the experiments of Minkoff and Damadian (7) with the modifications described above and analyzed the protein components of the complex by

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Abbreviations: EF-Tu, elongation factor Tu; DTT, dithiothreitol; $NaDodSO_4$, sodium dodecyl sulfate; Hepes, *N*-2-hydroxyethylpiperazine-*N*′-2-ethanesulfonic acid.

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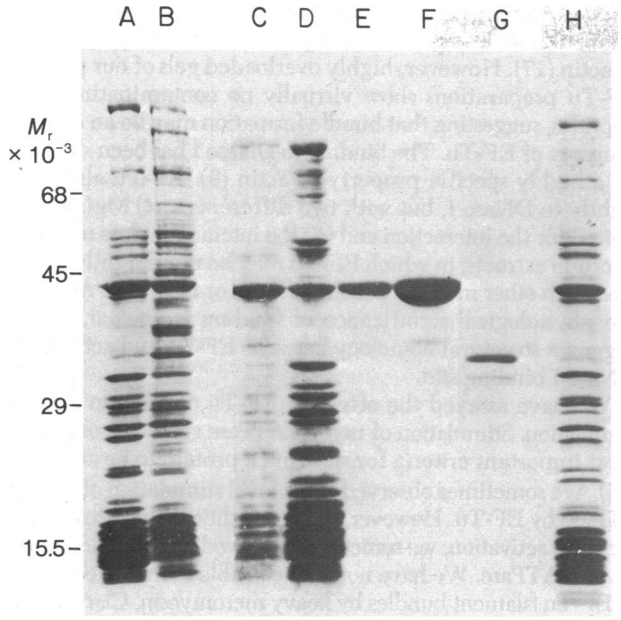

FIG. 1. $NaDodSO_4$/polyacrylamide gels. Lanes: A, polymerization complex; B, total cell protein; C, 9000 × *g* pellet of polymerization complex diluted into 0.6 M KCl/20 mM Hepes·NH_4OH/0.1 mM DTT for 2 hr at 4° and dialyzed overnight against 50 mM KCl/20 mM Hepes·NH_4OH/0.1 mM DTT at 4°; D, 9000 × *g* supernatant of same preparation; E, 9000 × *g* pellet of EF-Tu treated as in C; F, 9000 × *g* supernatant of same preparation; G, EF-Ts; H, polymerization complex.

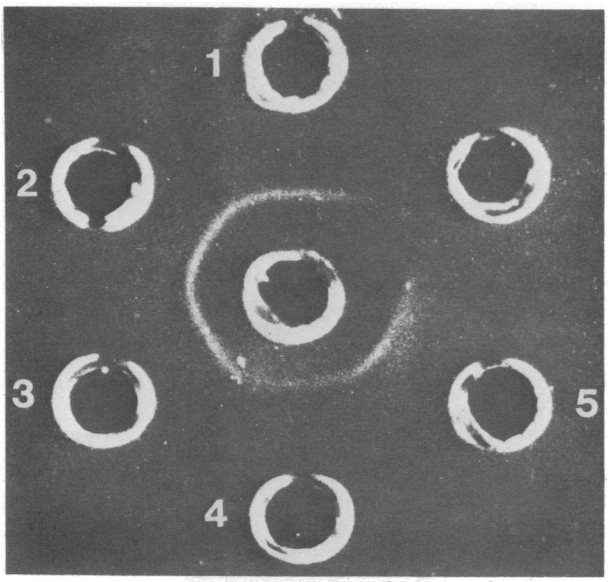

FIG. 2. Identification of EF-Tu by immunodiffusion. Agarose gels for double diffusion (15) contained 0.85% agarose/0.01 M Hepes·NH_4OH, pH 7.3/0.15 M NaCl/0.02% sodium azide. The center well contained rabbit anti-EF-Tu antibody. The other wells contained: 1, purified EF-Tu (*E. coli*); 2, polymerization complex (*E. coli*); 3, purified EF-Tu (*E. coli*); 4, polymerization complex (*Bacillus subtilis*; A. Jacobson, unpublished data); 5, polymerization complex (*E. coli*).

$NaDodSO_4$/polyacrylamide gel electrophoresis. Gels comparing the polypeptides of this complex to those of whole cells are shown in Fig. 1 (lanes A and B). The complex contained a large number of polypeptides, the most prominent of which had the same molecular weight as EF-Tu (42,000) and, in fact, comigrated with purified EF-Tu (Fig. 1, lanes E and F). Ouchterlony double-diffusion tests using anti-EF-Tu antibody showed a common precipitin band formed with either EF-Tu or the complex as the antigen (Fig. 2), thus confirming the presence of EF-Tu in the complex. Interestingly, the other protein synthesis elongation factor, EF-Ts, is virtually absent from the complex as shown by $NaDodSO_4$/gel electrophoresis (Fig. 1, lanes G and H).

The complex was treated with 0.6 M KCl in 20 mM *N*-2-hydroxyethylpiperazine-*N'*-2-ethanesulfonic acid (Hepes)·NH_4OH (pH 6.5) for 2 hr at 4°, dialyzed against 0.05 M KCl in the same buffer overnight, and subsequently centrifuged at 9000 × *g* for 30 min. Under these conditions, approximately 50% of the EF-Tu was preferentially precipitated from the complex as shown by gel analysis of the supernatant and pellet of such a preparation (Fig. 1, lanes D and C). Under the same conditions, approximately 25% of purified EF-Tu was sedimented. Significant increases in light scattering at 320 nm can be observed with both pure EF-Tu and the complex on addition of 0.6 M KCl (A. Jacobson, unpublished data). Thus, both purified EF-Tu and EF-Tu in the complex have similar aggregation properties. It is unknown whether the enhanced aggregation of EF-Tu in the complex reflects heterogeneity in the population of EF-Tu molecules or is due to its interaction with other proteins of the complex.

Affinity of EF-Tu for DNase I. Because EF-Tu can, under certain ionic conditions, undergo reversible polymerization, a property of actin-like proteins (16), we decided to determine whether EF-Tu has other actin-like properties. We measured the ability of EF-Tu to bind DNase I, because actin binds specifically to DNase I (9). We did not observe any bonding of pure EF-Tu to DNase I-Sepharose under the same conditions used by Lazarides and Lindberg (9). However, in the presence of 10 mM $MgCl_2$, virtually all the EF-Tu bound to DNase I-Sepharose and was eluted by 3.0 M guanidine·HCl (Fig. 3). Heat denatured EF-Tu and bovine serum albumin did not bind to DNAse-Sepharose under these conditions. Moreover, even in the presence of 10 mM $MgCl_2$, EF-Tu did not bind to the unsubstituted Sepharose 4B. In a crude extract from *E. coli*, there was virtually no binding of EF-Tu in either the presence or absence of 10 mM $MgCl_2$. Thus, our observations show that, although EF-Tu binds to DNase I, the binding differs from that of actin in that it requires $MgCl_2$ and does not occur in crude extracts. R. Storti has also observed that purified EF-Tu binds to DNase I but observed no Mg^{2+} requirement for binding (personal communication).

Ultrastructure. Many structural proteins, including actin, form filaments *in vivo* and can be induced to form filaments *in vitro* under various conditions. We therefore investigated the filament-forming ability of EF-Tu. Fig. 4 *A–D* shows representative electron micrographs of negatively stained structures of EF-Tu. These structures included loose aggregates (*A*), single filaments (*B*), and bundles of filaments (*C* and *D*). Bundles of filaments were found much more frequently than single filaments. Under the conditions cited in the figure legends, formation of individual filaments or bundles of filaments was not efficient. However, after overnight dialysis against 50 mM KCl/0.5 mM Tris·HCl, pH 7.5/0.2 mM $MgCl_2$/0.2 mM ATP/0.1 mM DTT (essentially the polymerizing conditions of Minkoff and Damadian), the majority of the protein in pure EF-Tu preparations formed aggregates with bundles of filaments (Fig. 4*E*). It should be noted that, due to the low buffering capacity of Tris at this molarity, the actual pH was 6.0.

We also studied by electron microscopy the interaction between EF-Tu and EF-Ts, the other protein synthesis elongation

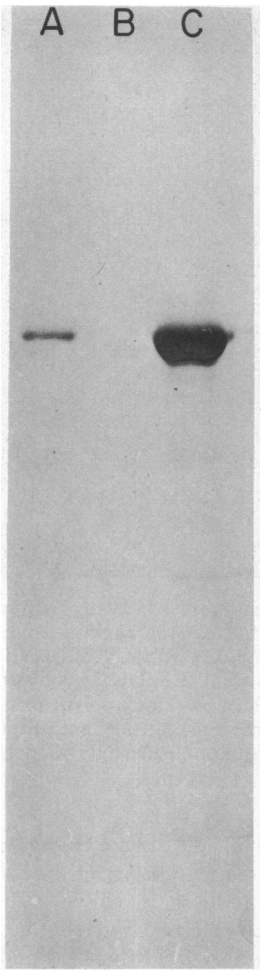

FIG. 3. DNase-Sepharose column chromatography of EF-Tu. Analysis by $NaDodSO_4$/polyacrylamide gel electrophoresis. Lanes: A, flow-through of 200 μg of EF-Tu applied to column in buffer I plus 10 mM $MgCl_2$; B, eluate with 0.3 M guanidine·HCl in buffer I plus 10 mM $MgCl_2$; C, eluate with 3.0 M guanidine·HCl in buffer I plus 10 mM $MgCl_2$.

factor to which EF-Tu binds. In the presence of EF-Ts, some of the EF-Tu in a pure preparation formed large bundles of filaments that differed from bundles formed in the absence of EF-Ts by their paracrystalline appearance (Fig. 4*F*). These showed a periodic repeat of 6 nm along the filament axis.

We also examined the ultrastructure of the polymerization complex. After treatment of the unpolymerized complex with 0.6 M KCl/20 mM Hepes·NH_4OH/0.1 mM DTT at pH 6.5 for 120 min at 4°, many bundles of filaments were seen arranged in a network-like array (Fig. 4*G*). These filament bundles were similar in appearance to those of purified EF-Tu (Fig. 4*E*).

DISCUSSION

We have investigated certain properties of the bacterial protein synthesis elongation factor EF-Tu, both in purified form and in a crude extract, that indicate that it may function as a structural protein within the bacterial cell. These properties are compatible with EF-Tu being an actin-like protein. For example, EF-Tu forms bundles of filaments under those conditions in which actin polymerizes to form single filaments. It is unclear whether the formation of filament bundles is an intrinsic property of EF-Tu or reflects the presence of a cross-linking protein contaminant that induces the formation of filament bundles as occurs with partially purified preparations of actin (17). However, highly overloaded gels of our purified EF-Tu preparations show virtually no contaminating polypeptides, suggesting that bundle formation may be an intrinsic property of EF-Tu. The binding to DNase I has been shown to be a highly specific property of actin (9). EF-Tu also binds tightly to DNase I, but with two differences: (*i*) $MgCl_2$ is required for the interaction and (*ii*) the interaction does not occur in crude extracts, in which EF-Tu may be more tightly associated with other molecules such as EF-Ts or tRNA (1). Although the physiological significance of binding is unclear, it does suggest a structural homology between EF-Tu and actin at the DNase I binding site.

We have assayed the effect of EF-Tu on myosin ATPase stimulation. Stimulation of myosin ATPase activity is one of the most important criteria for showing a protein to be actin-like (16). We sometimes observed a low level stimulation of myosin ATPase by EF-Tu. However, under conditions that are optimal for actin activation, we sometimes observed an inhibition of the myosin ATPase. We have not yet been able to show decoration of EF-Tu filament bundles by heavy meromyosin. Clarification of the interaction between EF-Tu and myosin will reveal whether EF-Tu is truly actin-like.

While we were preparing this manuscript, a paper by Rosenbusch and coworkers (18) appeared which compares structural and functional properties of EF-Tu and actin. Analysis of the 38,000 molecular weight fragment produced by partial tryptic digestion of both EF-Tu and actin showed similarities in certain portions of the two molecules; however, the homology did not appear to extend over large regions. They were unable to demonstrate either the polymerization of EF-Tu into filaments or the binding of EF-Tu to DNase I. However, the latter experiments were performed in the absence of Mg^{2+}. In this respect it should be noted that the polymerization of EF-Tu into filament bundles occurs only under certain conditions. In addition, we have preliminary evidence, based on microscopic observation, that large aggregates of EF-Tu may actually be composed of masses of filament bundles. The large aggregates appear as electron-dense masses unless they are previously disrupted by high salt concentration (B. Beck, unpublished data). The lack of binding of EF-Tu to DNase I may have been due to differences in the method used to detect such binding—i.e., their experiments were performed in the absence of $MgCl_2$ and used cosedimentation or coelectrophoresis as a measure of binding.

Although EF-Tu differs from actin in some respects, the two proteins may be related. Along these lines, we have calculated the SΔQ value, a statistical measure of relatedness between proteins based on amino acid composition (19, 20), for EF-Tu and actin. A value less than 50 is an indication of similarity between two proteins. We obtained a value of 40 for EF-Tu and actin, which substantiates the idea that these proteins may be evolutionarily related. The sequences of the two proteins may have diverged considerably but with retention of certain structural features, as may have happened with bacteriophage and egg white lysozymes (21). Resolution of the three-dimensional structures of EF-Tu and actin will be critical in further evaluating the relatedness of the two proteins.

The possibility that EF-Tu is a structural protein suggests that it has a role other than its function in protein synthesis. That EF-Tu might be a multifunctional protein has already been suggested by Miller *et al.* (22) based on the number of cellular components with which EF-Tu interacts. Brown and Blumenthal (23) have also suggested that EF-Tu might be a mul-

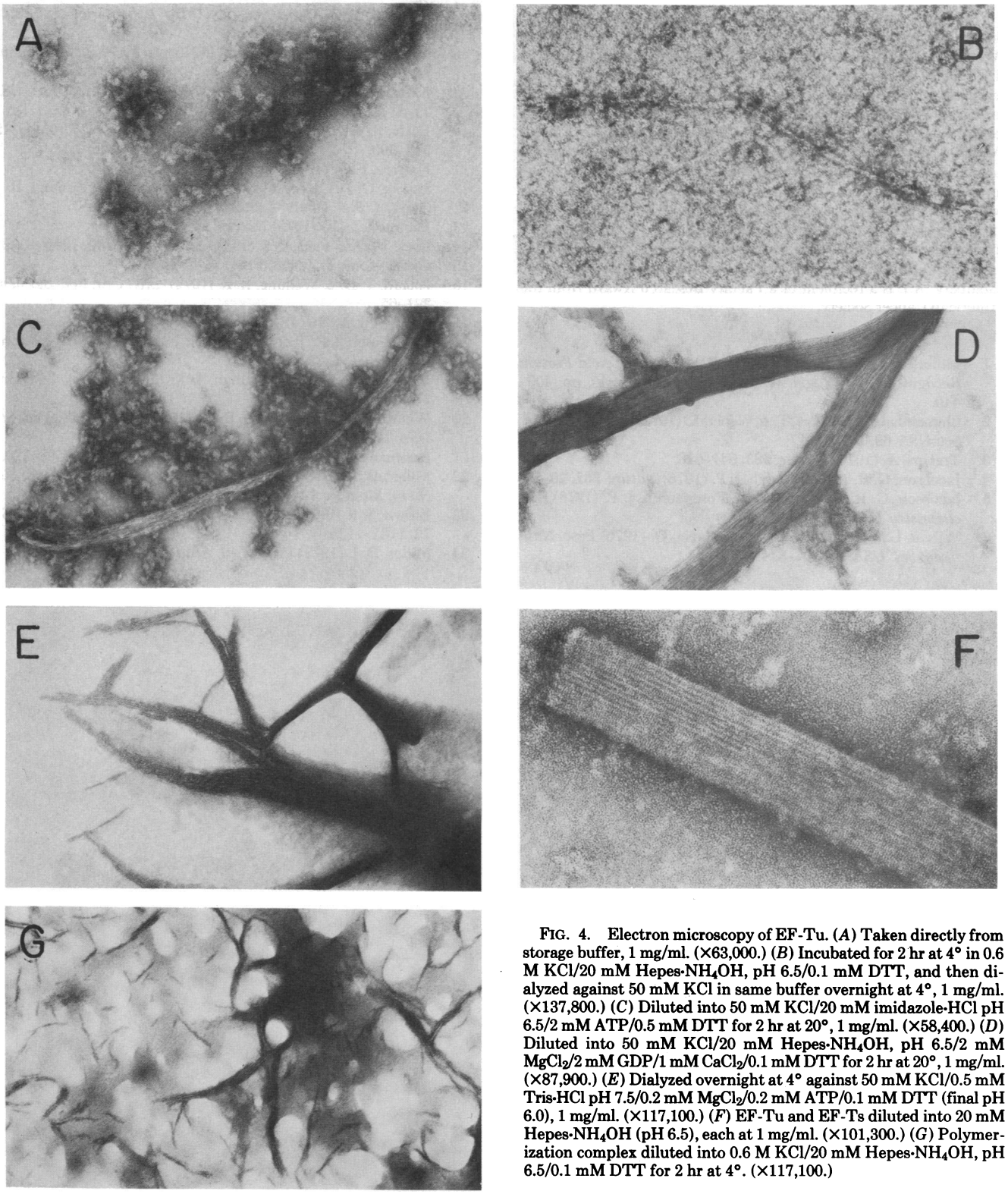

FIG. 4. Electron microscopy of EF-Tu. (*A*) Taken directly from storage buffer, 1 mg/ml. (×63,000.) (*B*) Incubated for 2 hr at 4° in 0.6 M KCl/20 mM Hepes·NH_4OH, pH 6.5/0.1 mM DTT, and then dialyzed against 50 mM KCl in same buffer overnight at 4°, 1 mg/ml. (×137,800.) (*C*) Diluted into 50 mM KCl/20 mM imidazole·HCl pH 6.5/2 mM ATP/0.5 mM DTT for 2 hr at 20°, 1 mg/ml. (×58,400.) (*D*) Diluted into 50 mM KCl/20 mM Hepes·NH_4OH, pH 6.5/2 mM $MgCl_2$/2 mM GDP/1 mM $CaCl_2$/0.1 mM DTT for 2 hr at 20°, 1 mg/ml. (×87,900.) (*E*) Dialyzed overnight at 4° against 50 mM KCl/0.5 mM Tris·HCl pH 7.5/0.2 mM $MgCl_2$/0.2 mM ATP/0.1 mM DTT (final pH 6.0), 1 mg/ml. (×117,100.) (*F*) EF-Tu and EF-Ts diluted into 20 mM Hepes·NH_4OH (pH 6.5), each at 1 mg/ml. (×101,300.) (*G*) Polymerization complex diluted into 0.6 M KCl/20 mM Hepes·NH_4OH, pH 6.5/0.1 mM DTT for 2 hr at 4°. (×117,100.)

tifunctional protein on the basis of their studies on EF-Tu as one of the components of the Qβ replicase.

What then could be additional functions of EF-Tu in a bacterial cell? Minkoff and Damadian (7) suggested that polymerization and depolymerization of a contractile protein are involved in the changes in cell volume that occur during uptake of potassium. The polymerization complex of a mutant deficient in potassium uptake polymerizes poorly under conditions in which the complex of the parent polymerizes well (7). It is also tempting to speculate that EF-Tu could also function in a structural way during protein synthesis in the movement of ribosomes relative to RNA. In light of results that indicate that EF-G, the elongation factor involved in translocation, excludes EF-Tu binding to ribosomes (24), such a function would depend on hitherto undetected interactions between EF-Tu and the ribosome.

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