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Bacteriophage Qβ Replicase Contains the Protein Biosynthesis Elongation Factors EF Tu and EF Ts

(RNA phage/transfer factors/ppGpp/renaturation)

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ABSTRACT **The enzyme, Qβ replicase, responsible for the replication of the RNA of *Escherichia coli* phage Qβ, is composed of four nonidentical subunits, three of which, I, III, and IV, are coded for by the bacterial genome, while subunit II is phage-specific.**

Subunit IV is shown to be identical to the protein synthesis elongation factor EF Ts by the following criteria: coelectrophoresis on polyacrylamide gels in sodium dodecyl sulfate and in urea buffers, identity of the first seven amino acids at the amino-terminus, precipitation of subunit IV by anti-EF T-factor serum, and stimulation of EF Tu-GDP exchange by subunit IV. Subunit III is shown to be identical to the protein synthesis elongation factor EF Tu by the following criteria: coelectrophoresis on sodium dodecyl sulfate gels, precipitation of EF Tu by anti-Qβ replicase serum, binding of guanine nucleotides, and binding of phenylalanyl-tRNA. In addition, Qβ replicase activity can be reconstituted from subunits I and II with EF Tu and EF Ts.

The RNA bacteriophage of *Escherichia coli*, Qβ, induces an enzyme, Qβ replicase, that is responsible for replication of the phage RNA. This enzyme has been extensively characterized and purified. It will copy Qβ RNA, but not the RNA of similar *E. coli* RNA phages. Early in its purification, it is capable of copying both phage RNA ("plus strands") and the RNA complement of the phage RNA ("minus strands"). The ability to copy "plus strands," however, is lost upon further purification. The purified core enzyme can be assayed with either "minus strands" or poly(C) as template. "Plus strand" activity can be restored by addition of host-coded factors (for a review, see Stavis and August, ref. 1).

Kamen (2) and Kondo, Gallerani, and Weissmann (3) found that the purified core enzyme consists of four nonidentical polypeptide chains of approximate molecular weights 70,000, 65,000, 45,000, and 35,000 (designated I, II, III, and IV in the nomenclature of Kamen). Subunit II is coded for by the phage genome, while the other three subunits are present in uninfected *E. coli*. The replicase of the serologically unrelated RNA phage f2 has been purified by Fedoroff and Zinder (4); it contains three host-coded polypeptides of similar, if not identical, molecular weights to those of Qβ replicase, in addition to the phage-coded subunit.

The four polypeptides of Qβ replicase can be separated into complexes of subunits I + II and subunits III + IV by incubation in a buffer of low salt concentration, followed by sedimentation on a low salt–glycerol gradient. Kamen (2) found that neither fraction alone shows activity in the poly-(C)-dependent assay, but partial activity could be recovered after the two were mixed together.

We report here that subunits III and IV of Qβ replicase are identical with EF Tu and EF Ts, respectively, two elongation factors identified as part of the mechanism of protein biosynthesis by Lucas-Lenard and Lipmann (5, 6).

Abbreviations: SDS, sodium dodecyl sulfate; Phe-tRNA, phenylalanyl-charged phenylalanine-tRNA.

RESULTS

For experiments with isolated subunits of Qβ replicase (from *E. coli* K12 strain Q13), the polypeptides were purified on sodium dodecyl sulfate (SDS)–polyacrylamide gels, eluted

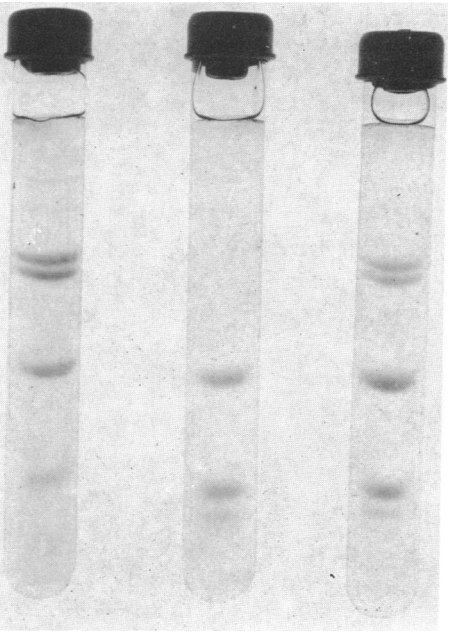

FIG. 1. 10% SDS–polyacrylamide gels were run according to the method of Weber and Osborn (24). The dye moved 11 cm. *Left* to *right*: Qβ replicase; EF Tu and EF Ts; and Qβ replicase and EF Tu + EF Ts. Purified Qβ replicase was prepared according to the method of Kamen (2), with an additional purification step. The pooled replicase-containing fractions from the phosphocellulose column were diluted to 0.1 M NaCl in the standard Tris buffer without Mg^{++}. The enzyme was chromatographed on a DEAE-Sephadex A50 column, and eluted with a 0.15–0.4 M gradient of NaCl. The replicase eluted at 0.2 M NaCl. The enzyme was then sedimented on the high salt–glycerol gradient for 36 hr.

1 3 5 7
Ala – Glx – Ile – Thr – Ala – Ser – Leu –

FIG. 2. The amino-terminal sequence of EF Ts and of subunit IV of Qβ replicase. Sequences were determined by the method of Weiner, Platt, and Weber (8). The subunits of Qβ replicase were separated on 10% SDS–polyacrylamide gels from which polymerization products had been eluted. After electrophoresis, protein bands were visualized by chilling the gels in the tubes at 4°. The band corresponding to subunit IV was cut out, eluted in 5 ml of water for 8–12 hr, and lyophilized.

and renatured according to Weber and Kuter (7). In other experiments, the complexes of subunits I + II and subunits III + IV were obtained from low salt–glycerol gradients as described by Kamen (2). Highly purified preparations of EF Tu and EF Ts from *E. coli* B were supplied by Drs. David Miller, John Hachmann, and Herbert Weissbach (Roche Institute of Molecular Biology, Nutley, N. J.).

Comparison of subunit IV and EF Ts

Subunit IV and EF Ts were not separable by electrophoresis either on 10% SDS–polyacrylamide gels (Fig. 1) or on pH 4.5 urea gels (not shown).

Using the SDS–dansyl(Edman) microtechnique of Weiner, Platt, and Weber (8), we sequenced the first seven amino acids at the amino-terminus of EF Ts and subunit IV. The sequences were identical (Fig. 2).

Antibody prepared against purified EF T-factor by Dr. Julian Gordon (also kindly supplied by Dr. Weissbach) forms precipitin lines of identity with EF Ts, Qβ replicase, and subunit IV on immunodiffusion plates (Fig. 3). The fact that this antibody does not react with EF Tu was noted by Gordon and Weissbach (27). No reaction was found with the other subunits of Qβ replicase.

The function assigned to EF Ts in protein biosynthesis is to facilitate removal of tightly bound GDP from EF Tu by formation of a EF Tu–EF Ts complex (6). A convenient assay for EF Ts, described by Weissbach, Miller, and Hachmann

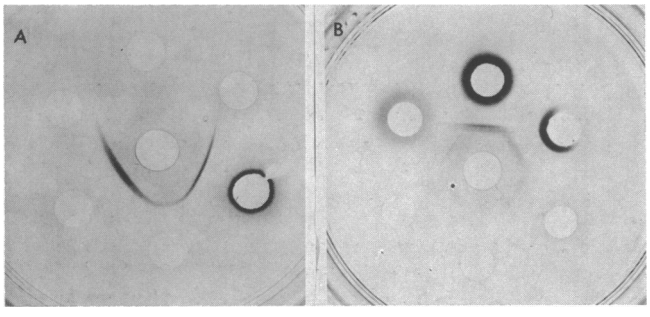

FIG. 3. Immunodiffusion. *Panel A:* Antiserum prepared against T-factor was placed in the center well. In the outside wells, *clockwise from top*: subunit III; subunits I + II; Qβ replicase; subunit IV; EF Ts; EF Tu. The amount of subunit IV used was about 25% of the amount of EF Ts and replicase. *Panel B*: Antiserum prepared against purified Qβ replicase was placed in the center well. In the outside wells, *clockwise from top*: Qβ replicase; EF Tu; subunits III + IV; blank; subunit IV; EF Ts. Purified subunits of Qβ replicase were obtained as described in the legend to Fig. 2. The sample was taken up in 8 M urea, and the SDS was removed by filtration through Dowex 1-X8 (7). Subunit III + IV complex was obtained with a low salt–glycerol gradient, as described by Kamen (2).

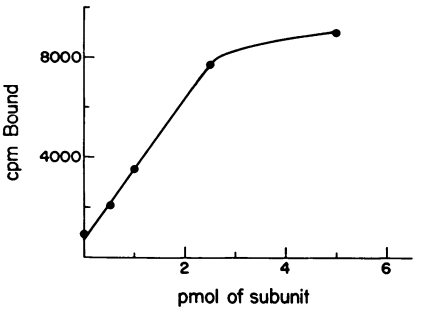

FIG. 4. EF Ts activity of Qβ replicase subunit IV. Pure subunit IV was obtained as described in the legend to Fig. 2, and renatured by a modification of the method of Weber and Kuter (7). Crystalline urea was added, to 8 M, to the solution containing subunit IV. After addition of 2-mercaptoethanol to 1%, the SDS was removed with Dowex 1-X8 (7), and the remaining solution was allowed to stand at room temperature for 2 hr. Subunit IV was then renatured as described in the legend to Table 3. The assays were performed according to Weissbach, Miller, and Hachmann (9). 44 pmol of EF Tu–GDP was used to start the reaction in each case.

(9), involves the exchange of labeled GDP with previously bound unlabeled GDP on EF Tu. Fig. 4 shows that subunit IV has EF Ts activity in this assay when mixed with EF Tu–GDP. We conclude that EF Ts and subunit IV are structurally and functionally identical.

Comparison of subunit III and EF Tu

EF Tu is known to form a complex with EF Ts (6). Since subunit IV appeared to be identical with EF Ts, and since subunits III and IV form a complex (2), it seemed reasonable that subunit III might be EF Tu.

EF Tu and subunit III coelectrophoresed on 10% SDS–polyacrylamide gels (Fig. 1). However, neither polypeptide formed a discrete band on pH 4.5 urea gels. We were unable to assign an amino-terminal residue to either subunit III or

TABLE 1. *Inhibition of GDP binding to subunits III + IV of Qβ replicase*

Competitor	Molar ratio: unlabeled/ labeled	% Inhibition of [^{3}H]GDP binding
Subunits III + IV		
GTP	10	13
GTP	100	44
GDP	10	93
ppGpp	10	71
ADP	200	14
EF Tu–GDP		
ppGpp	20	83

15 pmol of subunits III + IV or EF Tu–GDP was added to 0.1 ml of a buffer containing 0.05 M Tris–0.01 M Mg^{++} (acetate)–2 mM EDTA (pH 7.5) at room temperature. The indicated amounts of unlabeled competitor nucleotides were added, followed by [^{3}H]GDP to a final concentration of 1 μM (1770 Ci/mol). After a 5-min incubation at room temperature, the mixtures were filtered with suction through 6-mm nitrocellulose filters (Schleicher and Scheull B6) and rinsed with 0.1 ml of buffer. The filters were dried and counted in toluene–Omnifluor (Nuclear Chicago Co.) in a Beckman liquid scintillation counter.

TABLE 2. *Binding of Phe-tRNA to EF Tu and subunits III + IV of Qβ replicase*

	cpm [^{3}H]GTP bound	
	−Phe-tRNA	+Phe-tRNA
Experiment *1:*		
EF Tu	3115	776
III + IV	3055	1355
Experiment *2:*		
EF Tu	2712	1052
III + IV	3107	2080

A modification of the method of Weissbach, Redfield, and Hachmann (13) was used: 12 pmol of protein was mixed in 0.1 ml of a buffer containing 0.05 M Tris (pH 7.5), 0.01 M Mg^{++} (acetate), 2 mM EDTA, 1 mM phosphoenolpyruvate, 5 μg of pyruvate kinase, and 0.88 μM [^{3}H]GTP (5660 Ci/mol). The mixture was incubated at 37° for 5 min, then chilled to 0°. 13 pmol of Phe-tRNA was added, and the mixture was incubated at 0° for 5 min. The samples were filtered and counted as in Table 1.

EF Tu by means of the SDS–dansyl(Edman) technique. A similar result for EF Tu was previously obtained by Weissbach and coworkers (personal communication).

Fig. 3 demonstrates that antibody prepared against purified Qβ replicase forms precipitin lines on immunodiffusion plates with EF Tu, EF Ts, and Qβ replicase.

GDP is bound tightly by both Qβ replicase and by the complex of subunits III + IV. Details of these binding studies will be published elsewhere. The inhibition of GDP binding to subunits III + IV by various unlabeled competitors is shown in Table 1. GDP and ppGpp* (a gift of H. Ikeda and W. Haseltine) are most strongly bound. GTP binds about 100-fold less strongly, and ADP binds very weakly if at all. CTP, UTP, ATP, guanine, guanosine, and GMP do not inhibit the binding of labeled GTP, although dGTP does (data not given). EF Tu has similar binding characteristics: the binding of GDP is two orders of magnitude stronger than that of GTP (6). Table 1 shows that EF Tu also binds ppGpp strongly. We conclude that both EF Tu and subunits III + IV contain a binding site specific for guanine nucleotides.

EF Tu forms a ternary complex with aminoacyl-tRNAs and GTP (6). We were unable to do a parallel assay of complex formation by subunit III due to lack of active purified protein. However, assay of the subunit III + IV complex can be expected to yield similar results, since the presence of EF Ts does not interfere with aminoacyl-tRNA binding by EF Tu–GTP (10–12). Weissbach and coworkers have found that although the EF Tu–GTP complex will bind to nitrocellulose filters, the EF Tu–GTP–aminoacyl-tRNA complex passes through such filters. This observation is used to demonstrate binding of charged tRNAs to the EF Tu–GTP complex (13). Table 2 shows that by this criterion subunits III + IV bind Phe-tRNA. Since subunit IV is EF Ts, and since EF Ts does not bind GTP, the binding protein is subunit III. These experiments show that subunit III and EF Tu share the same function with respect to EF Ts binding, guanine nucleotide binding, and aminoacyl-tRNA binding.

* Guanosine 5′-diphosphate 3′- or 2′-diphosphate, [also called magic spot (M.S.) I]; see Cashel, M. & Kalbacher, B. (1970) *J. Biol. Chem.* **245**, 2309–2318.

Reconstitution of Qβ replicase activity

Kamen (2) has presented evidence that subunits III and IV are required for Qβ replicase activity. Table 3 shows experiments in which Qβ replicase is reconstituted after denaturation of separated subunits in 8 M urea, followed by renaturation in a buffer of high salt concentration. Control experiments with whole replicase give 20–30% recovery of input replicase activity. Subunits I + II show a low level of activity after renaturation, but if they are renatured in the presence of both EF Tu and EF Ts a high level of Qβ replicase activity is recovered. Neither EF Tu nor EF Ts alone will allow maximal recovery of activity. Subunits III + IV were denatured and renatured in the presence of subunits I + II, and gave a similar stimulation of activity. The amounts of activity recovered are within the expected range for the amount of protein added. We conclude that EF Tu and EF Ts can re-

TABLE 3. *Renaturation of Qβ replicase activity from urea-denatured separated subunits and from EF Tu and EF Ts*

Subunits present during renaturation	cpm [^{3}H]GMP incorporated
Experiment *1*:	
I + II	67
III + IV	16
EF Tu + EF Ts	0
I + II + III + IV	712
I + II + EF Tu + EF Ts	920
Qβ Replicase	2107
Experiment *2:*	
I + II	159
I + II + EF Tu	288
I + II + EF Ts	232
I + II + EF Tu + EF Ts	822
Experiment *3:*	
I + II	415
I + II + EF Tu	891
I + II + EF Ts	773
I + II + EF Tu + EF Ts	3667
Qβ Replicase	8964

Subunits I + II and subunits III + IV from low salt–glycerol gradients were denatured separately by the addition of urea, to a concentration of 8 M, and 2-mercaptoethanol, to 1%, and allowed to stand for 2 hr at room temperature. The solutions were renatured (after mixing when all four subunits were to be assayed) by dilution with seven volumes of a buffer containing 0.05 M Tris acetate, 0.01 M Mg^{++}(acetate), 1 mM EDTA, 0.15 M $(NH_4)_2SO_4$, 20% glycerol, and 1% 2-mercaptoethanol (26). When EF Tu and EF Ts were renatured with subunits I + II, a concentrated solution of the purified protein was added to the urea-containing subunit I + II solution 15 min before dilution in the renaturation buffer. All of the diluted mixtures were allowed to stand at room temperature for 4 hr before assay according to Kamen (2). About equimolar amounts of EF Tu + EF Ts and III + IV were used. In the experiments in which EF Tu or EF Ts alone were reconstituted with subunits I + II, twice as much of the single factor was added. The amount of subunits I + II was limiting in all experiments. The incorporation of ^{3}H in Experiments 1 and 3 are different because of unexplained differences in the level of renaturation, as shown by control experiments in which a constant amount of Qβ replicase was denatured and renatured.

place subunits III and IV in Qβ replicase, and that both polypeptides are necessary for maximal activity.

DISCUSSION

Subunit IV of Qβ replicase and the protein biosynthesis elongation factor EF Ts are identical by several criteria. The strongest evidence is that the sequence of the first seven amino-terminal aminoacid residues is the same. In addition, they have the same molecular weight and net charge. Furthermore, antibody prepared against EF T-factor precipitates subunit IV, and subunit IV reconstituted from SDS gels has EF Ts activity.

There is less physical-chemical evidence for the identity of Qβ replicase subunit III and protein biosynthesis elongation factor EF Tu. However, functional properties indicate identity. The molecular weights, as judged by SDS–polyacrylamide gels, are the same, and EF Tu crossreacts with immune serum prepared against purified Qβ replicase. Both bind EF Ts (assuming EF Ts and subunit IV are identical); they have similar properties with respect to guanine nucleotide binding: both bind GDP and ppGpp strongly, and GTP about 100-fold less strongly. Each protein also binds aminoacylated phenylalanyl-tRNA, as shown by inhibition of [^{3}H]GTP binding to nitrocellulose filters.

The essential experiment, however, was to show that Qβ replicase activity can be reconstituted from subunits I + II and both EF Tu and EF Ts. Thus, EF Tu and EF Ts can perform the functions of subunits III and IV in the poly(C)-dependent replicase assay. Both are necessary, since neither EF Tu nor EF Ts alone was sufficient to achieve maximal activity.

What function might EF Tu and EF Ts perform in the replicase molecule? The present view of the function of EF Tu in protein biosynthesis is that it transfers aminoacyl-tRNAs to the ribosome by means of a ternary complex containing EF Tu, GTP, and aminoacyl-tRNA. A GTP molecule is converted to GDP + Pi in the process of attachment, and EF Tu–GDP is released. EF Ts catalyzes the regeneration of EF Tu–GTP from EF Tu–GDP by formation of a EF Tu–EF Ts complex. GTP is then able to bind to the EF Tu–EF Ts complex, freeing EF Ts in the process (6). It is possible that EF Tu and EF Ts in Qβ replicase perform functions analogous to those just described. Both RNA phage RNAs and tRNAs have the sequence pCpCpA-OH at the 3′ end [reviewed by Cory, Spahr, and Adams, (14); Goodman *et al.*, (15)]. DeWachter *et al.* (16) have suggested that the nucleotide sequence of the 3′-terminal ends of RNA strands of phage RNA can be presented in a "tRNA-like" structure. One possible function for the elongation factors in the Qβ replicase molecule could be to bind the enzyme to the 3′ end of the RNA in order to initiate replication. Alternatively, EF Tu and EF Ts could also be involved in the RNA elongation reaction. If this were true, one might expect a molecule of GTP to be converted to GDP at every step. This possibility has been eliminated by our finding that as much as 87% of the input labeled GTP was incorporated into trichloroacetic acid-precipitable material in a poly(C)-dependent assay modified by omission of pyruvate kinase, phosphoenolpyruvate, and unlabeled GTP.

Travers *et al.* (17, 18) proposed that subunits III + IV (termed $\psi_r^{Q\beta}$) enable *E. coli* DNA-dependent RNA polymerase to synthesize ribosomal RNA, subject to inhibition by ppGpp. Our finding that these subunits bind ppGpp is consistent with this model. In this paper, we have shown that subunits III + IV are involved in protein biosynthesis. Therefore, if this model is correct, EF Tu and EF Ts perform functions in three biosynthetic processes: protein synthesis, stable RNA synthesis, and phage RNA synthesis. Recently, however, the experimental basis of the model of Travers *et al.* has been challenged (19–21). In any case, our finding that EF Tu binds ppGpp suggests directly that there may be some other involvement of EF Tu and/or EF Ts in the control of ribosomal RNA synthesis. The fact that EF Tu can distinguish charged from uncharged tRNA (6) further supports such a hypothesis, since the presence of an uncharged species of tRNA in a stringent cell appears to be the first "signal" for the cessation of ribosomal RNA synthesis (22) and accumulation of ppGpp (23).

We have shown that two proteins with well-documented roles in protein biosynthesis are also essential components of an RNA-dependent RNA replicase. This may be an example of a protein having a dual function, carrying out two different enzymatic activities. More likely, the protein has a single underlying function—which appears as a common step in two different biochemical processes. A deeper understanding of the synthetic process may reveal a single role for these proteins—such as positioning a nucleic acid correctly in order that a subsequent step can be performed.

We conjecture that this use of common components may be a more general phenomenon. EF Tu and EF Ts may themselves participate in other reactions, or other proteins will be found to participate in several complex processes. The remaining components of the Qβ replicase, subunit I, and the factors necessary for reading the plus strand may be further examples of such "multiuse proteins."

NOTE ADDED IN PROOF

We have found that the stimulatory activity of $\psi r^{Q\beta}$ for *in vitro* RNA synthesis on *E. coli* DNA (17), a 2-fold stimulation of total incorporation, can be duplicated by the use of EF Ts alone.

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