Defective 30S Ribosomal Subunits After Infection of *Escherichia coli* by T2 Ghosts

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Ribosomes from ghost-treated *Escherichia coli* can form in vitro initiation complexes but cannot translate added template RNA because of a defect in the 30S subunit; 16S rRNA is degraded.

Infection of *Escherichia coli* by T-even bacteriophages results in an immediate loss of capacity to induce new bacterial proteins (1, 2, 10, 12, 13, 16). New bacterial mRNA can be induced but is excluded from polysomes (11). Since the loss occurs so rapidly, it may result from the attachment itself, and be reproduced by DNA-less T-even ghosts (5, 7, 9).

Ribosomes from ghost-infected bacteria cannot translate exogenously supplied template RNA (14). One or both of the ribosomal sub-

units could be inactive (Table 1). When both subunits are from ghost-treated cells (line 2), the capacity to translate poly U is very low compared to that with normal subunits (line 1). The 50S subunits from ghosted cells are quite effective (line 3) but the 30S subunits (line 4) are completely inactive [30S and 50S refer to subunits, from ghost-treated cells, most of which are sediment at about 37S and 55S, respectively (Fig. 2)].

Note the background level of activity (lines 5

TABLE 1. Activities of 30S and 50S ribosomal subunits from uninfected and ghost-infected bacteria in polyphenylalanine synthesis^a

Set	Source		Additions					Counts/min ^b
	Ribosomes (70S)	S100	Uninfected			Infected		
I	None	Uninfected	1	+	+	-	-	8,990
			2	-	-	+	+	0
			3	+	-	-	+	4,740
			4	-	+	+	-	99
			5	+	-	-	-	565
			6	-	+	-	-	570
			7	-	-	+	-	256
			8	-	-	-	+	149
II	Uninfected	Uninfected	9	-	-	-	-	27,200
			10	+	-	-	-	23,900
			11	-	+	-	-	24,800
			12	-	-	+	-	22,500
			13	-	-	-	+	28,000
III	Infected	Infected	14	-	-	-	-	3,020
			15	+	-	-	-	25,960
			16	-	+	-	-	11,400
			17	-	-	+	-	6,690
			18	-	-	-	+	10,400

^a Each assay was in 50 μ liters and contained the components listed in reference (14) plus 10 μ g of poly U and the ribosome and S100 fractions indicated: uninfected or infected ribosomes (77 μ g) or S100 (58 μ g of protein); 30S subunits (50 μ g) or 50S subunits (32 μ g).

^b Average of duplicates which all agreed to within 10%. The incorporation with no poly U was determined for each combination and subtracted to give the recorded values. The controls (no poly U) were 1350 $\pm$ 300 counts/min in set I, 1,300 $\pm$ 50 counts/min in set II, and 2,800 $\pm$ 140 counts/min in set III.

FIG. 1. (A) Preparation of 30S and 50S ribosomal subunits from ribosomes of uninfected and 9-min T2-ghost infected *E. coli* B. Preincubated S30 extracts were centrifuged at $165,000 \times g$ for 2.5 h at 4 C. The pelleted ribosomes were resuspended in 10 mM Tris-hydrochloride, pH 7.5 + 10 mM magnesium acetate (TM) and dialyzed against 1 mM Tris-hydrochloride, pH 7.5, 60 mM KCl, 0.1 mM magnesium acetate, and 6 mM mercaptoethanol at 4 C. The subunits were separated by centrifugation at 24,000 rpm for 17 h in an International SB110 rotor through a 10 to 30% sucrose gradient made in the dialysis solution. Fractions were pooled to include 30 or 50S subunits and each pool was dialyzed for 3 h against TM plus 10 mM KCl, and then pelleted by centrifugation overnight at 48,000 rpm in an International A321 rotor at 4 C. Symbols: (—○—) uninfected; (—●—) ghost infected. (B) Sedimentation rates of ribosomal subunits in total extracts of uninfected and 2-min ghost-infected *E. coli* B. The latter culture was treated with T2 ghosts for 4 min with lysozyme present during the last 2 min. Both cultures had been exposed to [³H]uracil for more than two generations before chase with [³H]uracil to label only stable RNA. Metabolism was stopped by bringing cultures to 0 C with chloramphenicol (100 μg/ml) plus sodium azide (10 mM). The extracts were prepared in 10 mM Tris-hydrochloride, pH 7.4, plus 0.1 mM magnesium acetate and centrifuged at 4 C for 110 min through a 5 to 20% sucrose gradient in the same buffer at 45,000 rpm in a Spinco SW50 rotor. Symbols: (—○—) uninfected; (—●—) ghost infected. (C, D, and E) Acrylamide gel (2.7%) electrophoresis (3) of RNA from *E. coli* B, uninfected with lysozyme for 9 min (C); infected with T2 ghosts for 2 min plus 9 min with lysozyme (D), and 9 min plus 9 min with lysozyme present (E). The lysozyme (RNase free)-centrifugation steps enrich for the viable bacteria (14). The bacteria had been exposed to [³H]uracil for more than two generations to label total RNA. The RNA was subjected to 50 mA for 2.5 h at 4 C. The arrow indicates the bromophenol blue marker that was used to align the gels to each other.

to 8). Control ribosomes are not augmented by subunits (set II). In contrast, ribosomes from infected cultures give control activities when supplemented with normal 30S particles (line 15). Surprisingly, 50S particles also give some stimulation (line 16) although they are inactive alone (line 6).

Ribosomes from infected cells can complete translations in progress at the time of infection (endogenous activity) but cannot utilize added templates (14); we measured their competence to form initiation complexes with formyl-methionine-tRNA and MS2 RNA (Table 2). The translation initiation factors (IF) were partially purified (15) to measure their possible stimulatory effect if the ribosomes were defective in binding. Unwashed ribosomes and ribosomes washed in 1 M NH_4Cl were tested. Washed ribosomes have virtually no activity without IF (15). Ribosomes from ghost-treated bacteria appear normal with respect to forming initiation complexes with a natural mRNA (Table 2) and to binding poly U (14). Thus, the incapacity of these ribosomes does not result from a defect in the initial association but rather in polypeptide elongation.

There is a rapid decrease in content of 30S subunits after ghost infection (Fig. 1A) with subunits of about 37 and 55S size appearing in total cell extracts (Fig. 1A and B). These could be dimers and trimers of the 30S subunits (8). The aggregated forms are more resistant to RNase (8). Loss of 30S subunits could result

from loss of 16S rRNA (Fig. 1C, D and E), whereas there is no obvious loss of 23S rRNA or change in migration rates of either rRNA.

Whereas the usual concentration of the supernatant from the $100,000 \times g$ centrifugation (S100) from extracts of ghost-treated cells sup-

TABLE 2. Capacity of ribosomes from uninfected and ghost-infected bacteria to form initiation complexes with MS2 RNA^a

Source of ribosomes	Initiation factors	Ribosome treatment (counts/min) ^b	
		Unwashed	Washed
Uninfected	+	458 (100)	150 (33)
	-	168 (37)	3 (1)
Infected	+	474 (100)	160 (35)
	-	159 (35)	0 (0)

^a The reaction (4) contained in 100 μl iters: Tris-chloride (pH 7.8), 50 mM; NH_4Cl , 90 mM; MgSO_4 , 5 mM (TMN) plus GTP, 2 mM; 126 μg of unwashed or 100 μg of washed 70S ribosomes from uninfected or ghost-infected cells, 25 μg of MS2 RNA, [^3H]formyl-met-tRNA, about 4,000 counts/min, and 74 μg of partially purified initiation factors protein, if indicated. The reaction was at 30 C for 20 min and was stopped by chilling with addition of 3 ml of TMN and then collected on a 0.45- μm membrane filter (Millipore Corp.) and washed slowly with two 3 ml volumes of TMN (4).

^b The numbers in parentheses give the percent relative to uninfected unwashed ribosomes plus initiation factors. Omission of MS2 RNA or ribosomes gave 40 counts/min.

FIG. 2. The inhibition of *in vitro* polypeptide synthesis, after reaction for 30 min at 37 C (14), by S100 of extracts from T2 ghost-infected *E. coli* B. Increasing amounts, given as μg of protein in the S100, are added to: (A) 186 μg of normal ribosomes, 25 μg of MS2 RNA plus all components required for translation of natural mRNA (14); (B) S30 extracts (not preincubated to remove endogenous mRNA), from ghost-treated *E. coli* B containing 65 μg of ribosomes plus components for endogenous polypeptide synthesis; (C) preincubated S30 extracts from uninfected *E. coli* B, containing 98 μg of ribosomes, 25 μg of MS2 RNA plus all components required for translation of natural mRNA (14). In each case the effects of S100 from uninfected *E. coli* B are shown (—○—); S100 from ghost treated bacteria (—●—).

ports polypeptide synthesis at a normal rate, higher concentrations cause inhibition (14) (Fig. 2A). Uninfected S100 extract did not relieve the inhibition (not shown); the inhibitor is not competing with any normal component. Not all in vitro polypeptide synthesis is inhibited. Endogenous activity (no added mRNA) of S30 extracts from ghost-treated bacteria is close to normal (14), and is not inhibited by excess S100 (Fig. 2B) except for utilization of MS2 RNA (Fig. 2C). Poly U-stimulated phenylalanine incorporation was not decreased (not shown). The inhibitor fraction elutes with proteins from a DEAE column and is not active in the presence of concentrations of diethylpyrocarbonate which completely inhibit RNase activities (6). Whether the inhibitor is an RNase and is responsible for the rapid in vivo decay of the 16S rRNA remains to be seen.

In conclusion, ribosomes from ghost-treated cells can complete translations in progress, but cannot initiate new translations (14). This defect is here traced to the 30S ribosomal subunit but is not due to any incapacity to form initiation complexes. The 30S subunit and its 16S rRNA are being degraded during ghost infection. The presence of an inhibitor of in vitro translation of natural mRNA in the non ribosomal supernatant may be related to the rapid inactivation of the 30S particle in vivo.

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