Properties of Initiation-Free Polysomes of *Escherichia coli*†

Phang-Cheng Tai, Brian J. Wallace, Eugene L. Herzog, and Bernard D. Davis*

ABSTRACT: Purified polysomes were obtained from gently prepared lysates of *Escherichia coli* and from ribosomes incubated with phage R17 RNA, by gel filtration on Sepharose 4B. The endogenous polysomes incorporated amino acids several times as fast as the R17 viral polysomes. Most preparations lacked initiating activity: incorporation was complete by 10 min, and there was no response to added phage RNA unless initiation factors were added. Such polysomes are useful to distinguish antibiotic and other effects on peptide chain elongation from effects on an initiating system. For example, initiation was much more sensitive than elongation to inhibition by elevated Mg^{2+} or by lowered temperature.

Studies in peptide synthesis with natural messenger have made use either of bacterial extracts with added viral messenger (see review by Kozak and Nathans, 1972) or of gently prepared cell lysates containing endogenous polysomes (Staehelin *et al.*, 1963; Godson and Sinsheimer, 1967; Pestka and Hintikka, 1971). However, these systems support chain initiation as well as chain elongation, and to analyze mechanisms of interference with protein synthesis (*e.g.*, by antibiotics) it would be desirable to compare such preparations with a polysome preparation that carried out only chain elongation. Moreover, polysomes made with viral as well as with cellular messenger would be useful for comparing an initiating and a noninitiating system employing the same messenger (viral RNA), and also for verifying the relevance, for the cell, of antibiotic mechanisms observed with this messenger *in vitro*.

This paper will describe the preparation of active purified polysomes of *Escherichia coli* that lack initiation factors (IF),[1] and will compare the response of these preparations to changes in Mg^{2+} concentration and temperature with the response of an initiating system. The use of the initiating and noninitiating systems to study the actions of aurintricarboxylate, kasugamycin, and pactamycin will be described in the following paper (Tai *et al.*, 1973). Similar studies have revealed unexpected specific effects of streptomycin, spectinomycin, and erythromycin on an initiating system, which will be reported in subsequent publications.

Materials and Methods

Bacterial Strains and Growth. MRE600, an RNase I^- *E. coli* strain (Cammack and Wade, 1965), was used for the preparation of endogenous polysomes, supernatant fractions, and crude IF. For preparing viral polysomes *in vitro*, *E. coli* K12 strain s26 (Garen and Siddiqui, 1962) was used since S30 extracts (Nirenberg and Matthaei, 1961) of this strain formed more polysomes than did extracts of MRE600. Cells were grown at 37° with vigorous aeration in medium A (Davis and Mingioli, 1950) supplemented with $CaCl_2$ (5 mg/l.), $FeSO_4$ (250 μg/l.), glucose (0.4%), and Difco Casamino acids (0.2%). Exponential phase cultures (4×10^8 cells/ml) were rapidly chilled by pouring onto excess ice. The cells were then harvested by centrifugation, washed once with TKMD buffer (see below), and frozen as cell pellets with solid CO_2–acetone. These pellets were often stored at −76° until required for lysates.

Buffers. The standard buffer (TKM) contained 10 mM Tris-HCl pH 7.8, 50 mM KCl, and 10 mM $MgOAc_2$. Dithiothreitol, when added to the buffers, was at 1 mM (TKMD). For Sepharose gel filtration, and for storage of polysomes, glycerol (10 or 15%) was added to the buffer ($TKMDG_{10}$ or $TKMDG_{15}$).

Cell Lysis. (i) FREEZE–THAW–LYSOZYME. The method of Ron *et al.* (1966) was modified as follows. Lysozyme (4 mg) was added to 2×10^{11} cells in 2.5 ml of TKMD buffer. After two cycles of freezing and thawing with deoxycholate present the material could be stored at −76° until use. After thawing 50 μg of DNase was added and the lysate was clarified at 12,000*g* for 10 min. Deoxycholate was usually omitted because it solubilizes membranes, giving rise to turbid polysome preparations. However, without deoxycholate the polysomes were not any more active, and their yield was not always adequate.

(ii) GRINDING WITH SOLID CO_2. Cells were also lysed by grinding for 10–15 min in a mortar with repeated additions of powdered solid CO_2, which was then removed by sublimation at 0°. The resulting lysate, diluted with TKMD buffer (0.5 ml/g of frozen cells), was treated with DNase (10 μg/ml). The suspension was then centrifuged at 12,000*g* for 10 min to remove unlysed cells and debris. Purified polysomes from such lysates were generally somewhat more active than those obtained by the freeze–thaw method, presumably because the cell mass was frozen during the whole lysis period and no detergent was used. However, only 20–30% of the cells were lysed. In preparing radioactively labeled polysomes the freeze–thaw–lysozyme method was used because of the higher yield.

Chloramphenicol has often been used to prevent polysome runoff during lysis. However, we omitted this reagent, for the resulting polysomes were less active, by as much as 50%, even after gel filtration (as described below). Because of this omission the polysome content of the cell lysates prepared by either method described above was often low, ranging from 25 to 70% of the total ribosomes.

Sepharose Column Chromatography. Cell lysate (5.0 ml) (total 800–1000 A_{260} units) in $TKMDG_{15}$ buffer was applied to a column of Sepharose 4B (33 × 2.5 cm) equilibrated with $TKMDG_{10}$ buffer and eluted with the same buffer at 25–30 ml/hr. One-milliliter fractions were collected and their absorbance at 260 nm was measured. The polysome content of selected fractions was analyzed by zonal centrifugation as

† From the Bacterial Physiology Unit, Harvard Medical School, Boston, Massachusetts 02115. *Received October 16, 1972.* A preliminary account of part of this work has been presented (Tai *et al.*, 1972). This work was supported by grants from the National Institutes of Health and the American Cancer Society.

[1] Abbreviation used: initiation factors, IF.

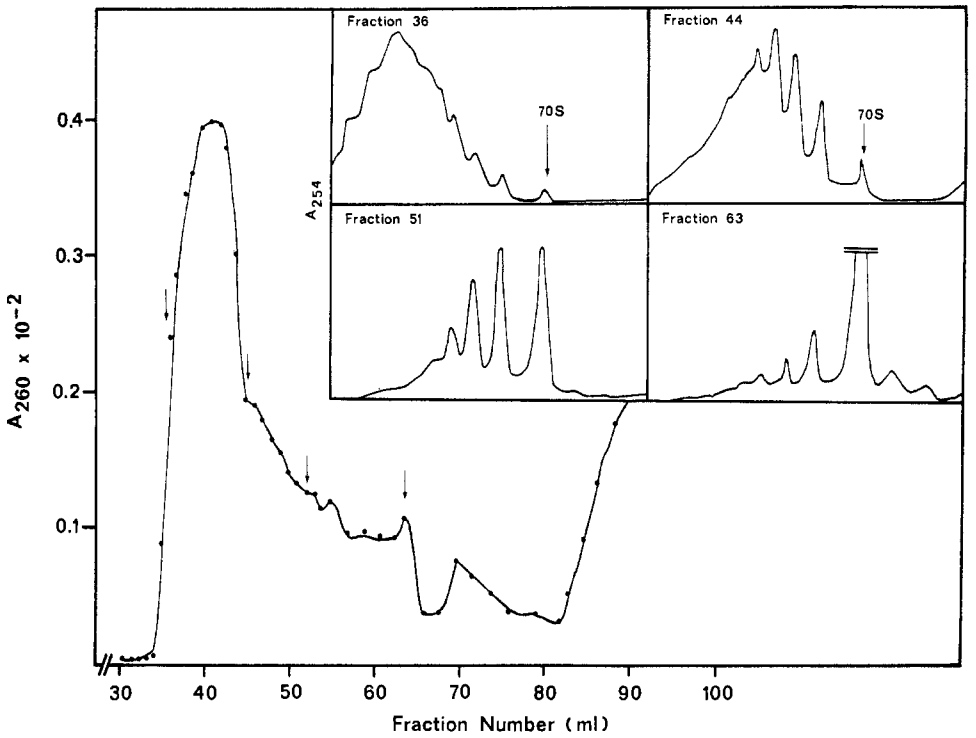

FIGURE 1: Separation of polysomes from 70S ribosomes and ribosomal subunits by gel filtration. Five milliliters of a cell lysate prepared by the freeze–thaw–lysozyme method and containing 1000 A_{260} units was applied to a Sepharose 4B column and eluted with $TKMDG_{10}$ buffer as described in Methods; inset, samples (containing approximately 60 μg of ribosomes) from fractions marked by arrows were analyzed for polysomes on sucrose gradients (see Methods).

described below. As a precaution against contamination with ribonuclease, all glassware and buffers were autoclaved.

Reagents. Phage R17 RNA (used as a messenger in some experiments) was prepared by the method of Gesteland and Boedtker (1964) except that phage was concentrated by using a mixture of polyethylene glycol 6000 and sodium dextran sulfate 500, as described by Albertsson (1967). "S100" supernatant was obtained by centrifuging S30 extracts at 235,000*g* for 3 hr. The top two-thirds was separated by careful aspiration, dialyzed against TKMD buffer for 3 hr, clarified (27,000*g* for 15 min), and stored in small portions at −76°. A crude mixture of IF was obtained from the first 1 M NH_4Cl wash of pelleted ribosomes by $(NH_4)_2SO_4$ precipitation (Iwasaki *et al.*, 1968). The NH_4Cl-washed ribosomes were activated by heating at 50° for 2 min (Grunberg-Manago *et al.*, 1969) in $TKMDG_{10}$ buffer.

[^{14}C]Valine was obtained from New England Nuclear Corp., DNase (RNase-free) from Worthington Biochemical Corp., and Sepharose 4B from Pharmacia Fine Chemicals, Uppsala, Sweden. Sparsomycin was a gift of the Cancer Chemotherapy National Service Center, Bethesda, Md., and kasugamycin was a gift of Bristol Laboratories.

In Vitro Protein Synthesis. Polypeptide synthesis was carried out in a reaction mixture (0.1 ml) containing 50 mM Tris-HCl pH 7.6, 60 mM NH_4Cl, 8 mM $MgOAc_2$, 2 mM dithiothreitol, 1 mM ATP-Tris, 0.02 mM GTP, 5 mM potassium phosphoenolpyruvate, 3 μg of pyruvate kinase, 0.03 mM [^{14}C]valine (70 μCi/μmol), 19 other amino acids at 0.05 mM each, 10 μl of "S100" extract, and either 50 μg of purified polysomes, 50 μg of NH_4Cl-washed ribosomes, or 25 μg of viral polysomes (assuming 16.6 A_{260} units = 1 mg). Phage R17 RNA (50 μg) and 20 μg of crude IF were added as indicated; these amounts yielded maximal activity. After incubation at 34° for the indicated time 1.0 ml of 5% trichloroacetic acid was added and the mixture was heated at 90° for 20 min, chilled, and filtered through Millipore (HA) filters and washed. These were then dried and counted in a Nuclear-Chicago low-background gas-flow counter, with a counting efficiency of 12%. The molarity of the [^{14}C]valine was verified by isotope dilution.

Zonal Analysis. Samples, usually containing 50–100 μg of ribosomes, were layered onto chilled 4-ml linear 5–35% sucrose gradients, in TKM_5 buffer with an 0.8-ml cushion of 45% sucrose. Fifty microliters of 0.2% chloramphenicol was usually layered on simultaneously to prevent further polysome runoff (Modolell and Davis, 1968). Gradients were centrifuged at 45,000 rpm in a Spinco SW50.1 rotor at 3° and then scanned for 254-nm absorption in an Isco gradient analyzer. Free ribosomes were estimated by centrifugation in sucrose gradients containing 60 mM Na^+ rather than 50 mM K^+ ($TNa_{60}M_5$).

Results

Purification of Polysomes. Cells were lysed either by the freeze–thaw–lysozyme method or by grinding with solid CO_2 (see Methods) and were subjected to gel filtration on Sepharose 4B. With a typical preparation a well-defined peak, representing approximately 40% of the total A_{260}, eluted between fractions 36 and 44 (Figure 1). Zonal centrifugation showed that this peak was rich in polysomes (Figure 1, inset), the average length decreasing in the later fractions of the peak. The total peak contained less than 5% 70S ribosomes, and no subunits could be detected even in its last fraction (fraction 44). The percentage of 70S ribosomes rose in later fractions: fraction 63 (Figure 1, inset) consisted mainly of 70S ribosomes together with some 30S and 50S subunits. Hence, the sub-

TABLE I: Effect of Some Antibiotics on Peptide Synthesis by IF-Free Polysomes.[a]

Antibiotics	Concn (μg/ml)	% Inhibition
Sparsomycin	100	95
Chloramphenicol	100	93
Puromycin	100	99
Fusidic acid	100	95
Tetracycline	235 (0.5 mM)	97

[a] Purified polysomes were incubated for 10 min at 34° in a peptide-synthesizing system containing various antibiotics, and the incorporation of [^{14}C]valine was measured (see Methods). The incorporation in a tube without antibiotic (3937 cpm) was taken as 100% activity. Charging of tRNA with amino acids was not affected by any of the antibiotics at the concentration used (data not shown).

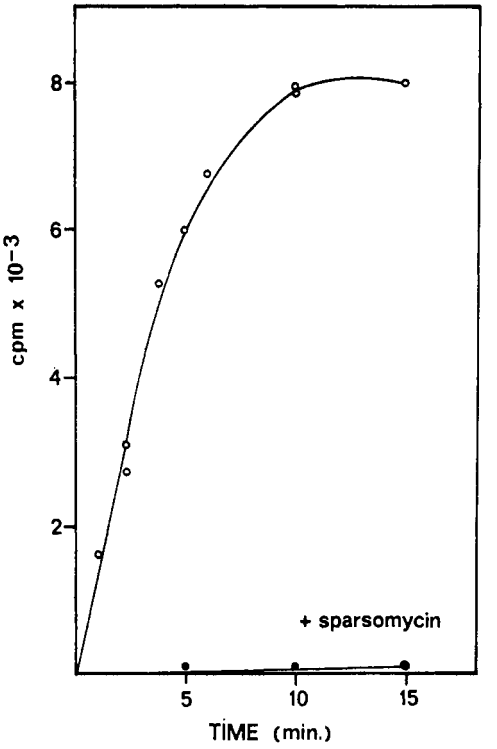

FIGURE 2: Kinetics of polypeptide synthesis on purified polysomes. Purified polysomes were incubated in a peptide-synthesizing system containing [^{14}C]valine, and the incorporation at the times specified was determined (see Methods). Sparsomycin (125 μg/ml) was included in a parallel set of tubes. Assuming that the protein formed contains 5.5% valine, like total *E. coli* protein (Roberts *et al.*, 1955), and that every ribosome is active, the average amino acid incorporation time at 34° was calculated as follows: in the reaction mixture 50 μg of ribosomes (mol wt 2.7×10^6) incorporated in 1 min 1600 cpm of valine, of specific activity 18.4×10^6 cpm/μmol. Hence $(1600/18.4 \times 10^6) \times (2.7 \times 10^6/50) \times (100/5.5) = 85$ amino acids/min or 1.5 amino acids per second per ribosome. Total incorporation, by 10 min, amounted to 8016 cpm of valine, or $(8016/1600) \times 85 = 425$ amino acids per ribosome.

units are well separated from the polysomal ribosomes. Fraction 88 contained predominantly supernatant proteins and tRNA.

The fractions containing large polysomes (as in Figure 1, fractions 36–42) were pooled and stored at −76° in small quantities until used. In subsequent preparations fractions from the first half of the large initial A_{260} peak were pooled and used as a purified polysome preparation without assaying the individual fractions for polysome content. The polysomes were found to be stable to stirring in a vortex mixer, pipetting, centrifugation, and several cycles of freezing and thawing. They could be stored at −76° in $TKMDG_{10}$ for several weeks without loss of polymerizing activity.

Prior to developing the chromatographic method we purified polysomes by centrifuging a clarified cell lysate through a glycerol gradient (5–35% in TKMD buffer), for sufficient time to pellet the polysomes but few 70S ribosomes and subunits. Such polysomes were less active in peptide synthesis (*ca.* 50%) than those prepared by gel filtration, and most preparations contained appreciable initiating activity. However, the method has been useful in preparing small amounts of relatively IF-free active polysomes labeled with heavy atoms (Subramanian and Davis, 1973).

Kinetics of Peptide Bond Formation. When purified polysomes were incubated at 34° with "S100" supernatant, amino acids, and an energy source (see Methods) there was considerable incorporation of amino acids. As Figure 2 shows, synthesis was linear for 3–4 min, at 1.5 amino acids per second per ribosome (for calculation see legend), and by 10 min incorporation had ceased. The total incorporation in this preparation corresponded to 425 amino acids per ribosome. As expected, both the initial rate and the total extent of [^{14}C]-valine incorporation were proportional to the concentration of polysomes over the range of 250–1000 μg/ml.

Incorporation was inhibited completely by sparsomycin (Figure 2), or by several other inhibitors of polypeptide chain elongation (chloramphenicol, fusidic acid, tetracycline, or puromycin, Table I). Hence the incorporation clearly reflects protein synthesis by the endogenous polysomes.

Conversion of Polysomes to Free Ribosomes. As Figure 3b shows, after 5 min only 25% polysomes, mostly very short, remained, and at 10 min only 10%. This conversion was due primarily to runoff and not to attack by ribonuclease, for with sparsomycin present to block chain elongation the polysome profile changed only moderately (Figure 3d), just as when purified polysomes were incubated in TKMD buffer. Moreover, the 70S ribosomes that accumulated during protein synthesis were mostly dissociated by the physiological dissociation factor (Figure 3e) or by centrifugation in a sucrose gradient containing Na^+ rather than K^+ (Figure 3f); these procedures dissociate free but not complexed ribosomes (Subramanian *et al.*, 1969; Beller and Davis, 1971). Finally, when a preparation was incubated with radioactive amino acids until incorporation was essentially complete (10 min) 71% of the acid-precipitable radioactivity was found to sediment above the 70S peak (results not shown).

Lack of Initiation Factor Activity in Polysome Preparations. The short duration of protein synthesis with these purified polysomes suggested that it occurred only by extension of already initiated peptide chains, with little reinitiation by released ribosomes. The absence of reinitiation is evidently due to lack of IF. Thus, addition of IF at the start of the incubation increased activity 10–30%. Furthermore, this stimulation was abolished by addition of 200 μM kasugamycin, which blocks initiation on added viral mRNA but does not inhibit chain elongation with purified polysomes (Tai *et al.*, 1973). Finally, following runoff the addition of phage RNA led to no significant incorporation, but if optimal IF was also

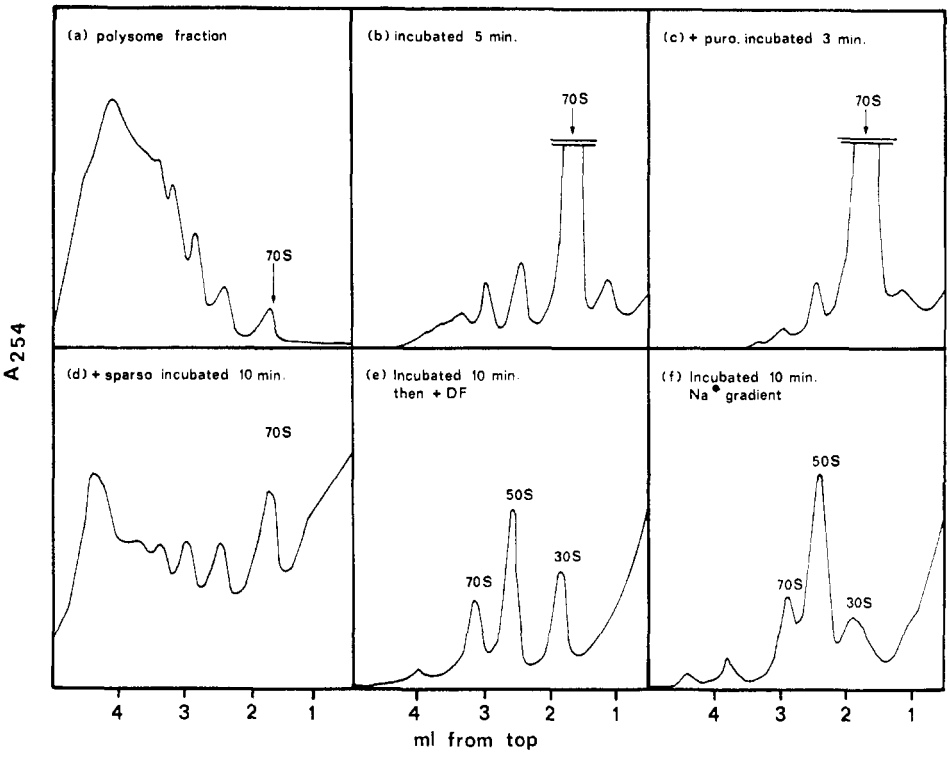

FIGURE 3: Conversion of purified polysomes to free ribosomes. Purified polysomes were incubated for peptide synthesis, with additions as indicated. Samples were chilled and analyzed for polysomes on sucrose gradients (see Methods), which were sedimented for 45 min (a–d) or for 60 min (e, f): (a) purified polysomes (in buffer, no incubation); (b) 5-min incubation followed by the addition of chloramphenicol (100 μg/ml) to prevent further runoff; (c) incubation with puromycin (200 μg/ml) for 3 min; (d) incubation with sparsomycin (100 μg/ml) for 10 min; (e) incubation for 10 min, followed by the addition of 100 μg of crude IF (as a source of dissociation factor) and further indubation for 10 min; (f) incubation for 10 min, then analysis in a $TNa_{60}M_5$ gradient rather than a TKM_5 gradient as used in a–e.

added the activity was at least as great as that observed with either an S30 extract or with NH_4Cl-washed ribosomes (Table II).

These results incidentally suggest that the "S100" used was virtually free of IF activity, as expected from the careful elimination of native subunits (see Methods). Moreover, with this "S100" a preparation of six-times washed ribosomes was inactive with added R17 RNA unless IF was also added (Table II, line 3).

Initiation onto Endogenous mRNA. The stimulatory effect of added IF with the endogenous polysome preparations suggests that runoff ribosomes can reinitiate appreciably onto endogenous mRNA, even in a dilute system (0.5 mg/ml of polysomes), provided IF is present. Indeed, some preparations of purified polysomes evidently contained an appreciable amount of IF, for after runoff they exhibited up to 30% as much activity with added phage RNA as was observed with an optimal addition of IF. Moreover, the endogenous incorporation by these preparations was reduced 10–25% by aurintricarboxylate or kasugamycin, at concentrations that selectively block initiation (Tai *et al.*, 1973); the same addition had no effect on IF-free preparations. The source of the IF in the reinitiating preparations is unknown; no subunits (and less than 5% 70S ribosomes) were detected, but the polysomes may have contained initiation complexes.

Of the 11 polysome preparations tested seven had IF contents (when tested with phage RNA) of only 1–10% of the optimum. These "IF-free" polysomes were used for all later experiments on chain elongation.

Preparation of IF-Free Polysomes on Viral RNA. To prepare similar IF-free polysomes with phage RNA as messenger a preincubated S30 extract was incubated with phage R17 RNA, as described in the legend to Figure 4; this treatment converted 60% of the ribosomes into disomes, trisomes, and tetrasomes. On passage through a Sepharose 4B column the first 30% of the ribosomal particles recovered was found to consist almost entirely of short polysomes, with only a small 70S peak and no visible subunits (Figure 4b). These purified viral polysomes incorporated amino acids at an initial rate of 18 per ribosome per minute; the total incorporation in 20 min was 97 amino acids per ribosome (for calculation see Figure 6 legend, below). IF was evidently absent, for the addition of R17 RNA caused no further incorporation unless IF was also added (Table III).

Effect of Mg^{2+} Concentration and Temperature on Amino Acid Incorporation. With active IF-free polysomes the effects of Mg^{2+} concentration and temperature on chain elongation can be studied without interference from initiation. As Figure 5 shows, with endogenous polysomes (measured after 3 or 10 min), and with viral polysomes, Mg^{2+} exhibited a broad optimum, 8–12 mM; even at 20 mM the incorporations were 40% of the maximum, though at high Mg^{2+} activity varied somewhat from preparation to preparation and increased during storage. In contrast, initiation-dependent incorporation, with NH_4Cl-washed ribosomes plus IF and phage R17 RNA, had a much narrower Mg^{2+} range: the minimal Mg^{2+} requirement was similar, but activity fell off rapidly above 8 mM Mg^{2+} and virtually disappeared at 20 mM.

The two systems also differ in their sensitivity to cold. As Figure 6 shows, the rate of amino acid incorporation by endogenous polysomes varied only moderately between 25 and 42°, and at 14° it was still one-third of the maximum; even at

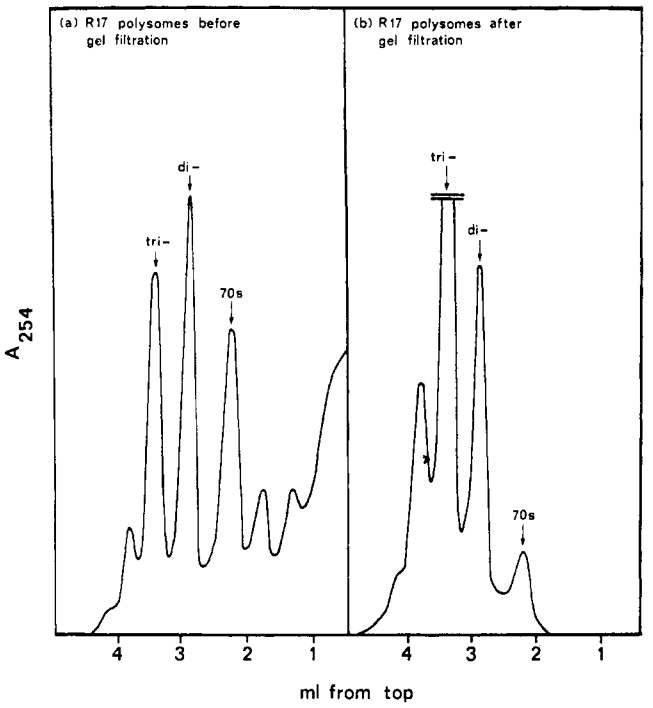

FIGURE 4: Preparation of IF-free phage R17 polysomes. An S30 extract of *E. coli* K12 (containing 12 mg of ribosomes) was incubated with 0.5 mol equiv of phage R17 RNA, in 2.0 ml of a peptide-synthesizing mixture (as in Methods except that the valine was unlabeled). After incubation at 34° for 8 min the reaction mixture was rapidly chilled to 0° and was chromatographed on Sepharose 4B (1.5 × 18 cm) as previously described. Fractions (0.5 ml) were collected, the A_{260} was measured, and the first four fractions with significant absorbance were pooled. A sample of the pooled fractions and a sample taken before gel filtration were analyzed for polysomes (see Methods).

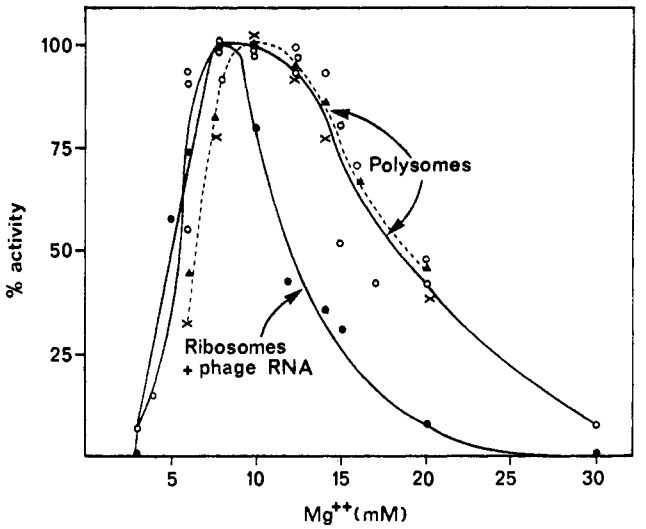

FIGURE 5: Effect of Mg^{2+} concentration on peptide synthesis with and without initiation. Reaction mixtures (100 μl), adjusted to the Mg^{2+} concentration indicated, contained IF-free endogenous polysomes (○), or IF-free phage R17 polysomes (▲), or 1× NH_4Cl-washed ribosomes plus phage RNA and optimal IF (●), in addition to the other reagents required for peptide synthesis (see Methods). Incubation was at 34° for 10 min with endogenous or phage polysomes or for 30 min with NH_4Cl-washed ribosomes, or for 3 min with endogenous polysomes (×).

0° there was detectable incorporation. With the initiation-dependent system, in contrast, the rate of synthesis was considerably decreased at 25° and at 14° it was negligible.

Initiation also showed a more striking response to elevated temperatures. With the initiating system incorporation increased gradually at the start (as expected from the increasing number of polysomes), and this increase was faster at 42° than at 34° (Figure 6c). With the system engaged in pure chain elongation, which exhibited no lag, the rate was similar at the two temperatures (Figure 6a).

Discussion

This paper describes a rapid, simple, and reproducible procedure for preparing purified endogenous polysomes from cell lysates of *E. coli*. Cells were lysed, either by a modification of the freeze–thaw–lysozyme technique or by grinding with powdered solid CO_2, and the lysates were filtered through a Sepharose 4B column. The early fractions (Figure 1) were found to be rich in larger polysomes (as also observed by Hirashima and Kaji, 1970). The first two-thirds of the polysome peak contained less than 5% 70S ribosomes and no detectable subunits. The polysomes were active in peptide synthesis but only for a short period: by 10 min incorporation was complete and the polysomes were almost entirely converted to free ribosomes, as shown by the loss of nascent peptide and by their dissociation by Na^+ or by the dissociation factor (Figure 3).

The short duration of polypeptide synthesis with these prep-

TABLE II: Lack of IF Activity in Purified Endogenous Polysomes and in "S100" Extract.[a]

	[^{14}C]Valine Incorporated (cpm) after Preincubation			
	Additions			
Ribosomes	None	Phage R17 RNA	Phage R17 RNA plus IF	% IF Activity[b]
1. Polysomes (prep 1)	294[c]	380	6000	1.6
2. Polysomes (prep 2)	231	630	7860	5
3. 6× washed ribosomes	50	73	2544	1
4. 1× washed ribosomes	63	1278	7168	17
5. S30 extract	100	6170		

[a] Purified endogenous polysomes were allowed to run off by incubation in a peptide-synthesizing system (0.02 mM nonradioactive valine) for 10 min at 34°. Similar amounts of NH_4Cl-washed ribosomes and an S30 extract were treated in the same way. [^{14}C]Valine was added, together with excess phage R17 RNA and optimal crude IF as indicated (see Methods). The reaction mixture was further incubated for 30 min and incorporation of [^{14}C]valine was determined. For NH_4Cl washing, ribosomes were treated with 1 M NH_4Cl (one time washed ribosomes) as described in Methods. The procedure of Lucas-Lenard and Lipmann (1966) was then followed to obtain six times washed ribosomes. [b] % IF activity is: (cpm stimulated by addition of R17 RNA/cpm stimulated by addition of R17 RNA plus IF)100. [c] Incorporation by polysomes during preincubation was 3937 cpm measured in a separate tube.

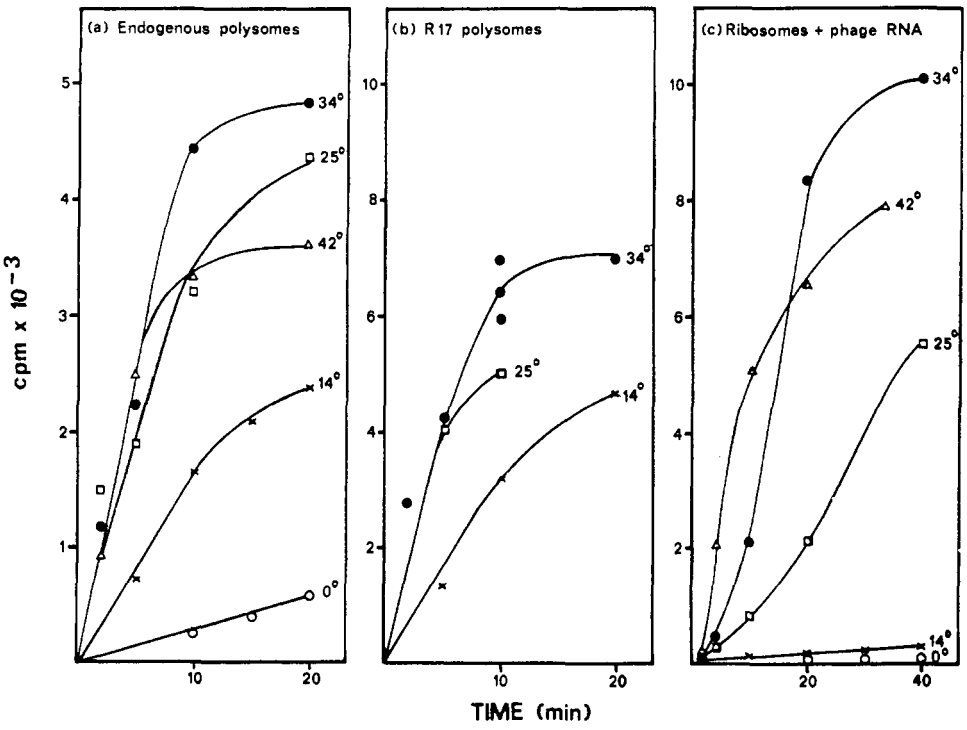

FIGURE 6: Effect of temperature on the kinetics of polypeptide synthesis with and without initiation. The rate of [^{14}C]valine incorporation was measured at various temperatures, with conditions otherwise as in Figure 5; Mg^{2+} was 8 mM: (a) IF-free endogenous polysomes; (b) IF-free phage R17 polysomes; (c) NH_4Cl-washed ribosomes plus phage R17 RNA plus IF. R17 polysomes (25 μg) in 1 min at 34° incorporated 1318 cpm of valine, of specific activity 55.7×10^6 cpm/μmol. Since the predominant product, coat protein of phage R17, contains 13.7% valine (Weber, 1967), the rate was $(1318/55.7 \times 10^6) \times (2.7 \times 10^6/25) \times (100/13.7) = 18$ amino acids per minute per ribosome. Total incorporation, by 20 min, amounted to 6917 cpm of valine, or $(6917/1318) \times 18 = 97$ amino acids per ribosome.

arations (<10 min at 34°) suggested chain completion without reinitiation. Indeed, with most preparations the runoff ribosomes could not initiate significantly on added viral RNA. The cause is evidently lack of IF, for its addition restored a high level of activity (Table II). Moreover, a specific inhibitor of initiation, kasugamycin, blocked this additional incorporation, though it does not inhibit synthesis by the IF-free polysomes (Tai *et al.*, 1973). Incorporation of amino acids by IF-free polysomes thus represents chain elongation by ribosomes past the stage of initiation. Polysomes prepared on viral RNA were purified similarly (Figure 4) and were also found to be free from initiating activity (Table III); they have been particularly useful as controls for an initiating system employing the same messenger.

TABLE III: Lack of IF Activity in Purified Phage R17 Polysomes.[a]

Additions	[^{14}C]Valine Incorporated (cpm)	% IF Activity
None	250	
+ R17 RNA	321	2.4
+ IF + R17 RNA	3219	100

[a] Reaction mixtures (0.1 ml) were as in Table II except that 0.01 mM valine and 25 μg of R17 polysomes were used. Polysomes were allowed to run off at 34° for 20 min prior to the addition of [^{14}C]valine plus viral RNA and IF as indicated. After incubation for a further 20 min the incorporation of [^{14}C]valine was determined.

The initial rate of chain elongation by the endogenous polysomes at 34° was 85 amino acids per minute per ribosome. This rate is only one-tenth that observed in cells (Maaløe and Kjeldgaard, 1966; Forchhammer and Lindahl, 1971), but it is several times that observed *in vitro* with viral polysomes: 18 amino acids per minute per ribosome in the present work (Figure 2), or 25 amino acids with viral RNA being translated in a less purified (S30 extract) system (Modolell and Davis, 1968; Webster and Zinder, 1969). In contrast, with a system that simultaneously transcribed and translated phage T4 DNA *in vitro*, forming lysozyme, synthesis proceeded at the much higher rate of 180 amino acids per minute per ribosome at 31° (Wilhelm and Haselkorn, 1970). Those authors suggested that the low rate of translation of viral RNA, compared to freshly transcribed T4 messenger, may be due to restriction of movement of the ribosomes by the secondary structure of the viral RNA. Secondary structure could also account for the intermediate rate observed with endogenous polysomes, but other factors, such as differences in punctuation and in the state of the ribosomes, cannot be excluded.

Chain elongation, on both endogenous and viral polysomes, is much less sensitive to elevation of Mg^{2+} concentration than is initiation (*i.e.*, synthesis by NH_4Cl-washed ribosomes with viral RNA and IF added). Thus the rate of elongation was relatively constant between 8 and 12 mM Mg^{2+}, and fell only to 40% of the maximum at 20 mM, whereas initiation had a similar Mg^{2+} *minimum* but fell off rapidly with increasing Mg^{2+} above a sharp optimum at 7–9 mM (Figure 5). It is not known whether the decreasing chain elongation at higher

Mg^{2+} concentrations is due to a direct effect on some step in this process or to an effect on termination and release. Flessel (1971) has also observed a rather broad Mg^{2+} optimum for endogenous *E. coli* polysomes, isolated from a sucrose gradient.

Chain elongation is also much less sensitive than initiation to lowering the temperature: at 14° elongation was one-third as rapid as at 34°, but initiation was negligible (Figure 6). This *in vitro* demonstration confirms a conclusion already indicated by the finding that bacterial cells incubated at 0° convert their polysomes into ribosomes (Goldstein *et al.*, 1964; Das and Goldstein, 1968; Oppenheim *et al.*, 1968). (These experiments were the basis for the use of slow cooling for accumulating runoff ribosomes in cells: Algranati *et al.*, 1969; Subramanian *et al.*, 1969.) The reaction that limits initiation under both these unfavorable conditions may well be the dissociation of runoff ribosomes by IF3, for this step in initiation (reviewed in Davis, 1971) is markedly inhibited by even moderate Mg^{2+} concentrations (Subramanian and Davis, 1970) and by low temperature (Subramanian *et al.*, 1968; Albrecht *et al.*, 1970).

Total incorporation in our most active preparation of IF-free endogenous polysomes corresponded to 425 amino acids per ribosome (Figure 2 legend); the values in other preparations ranged from 200 to 400. This large value was surprising, for such preparations are presumably completing chains already half-synthesized on the average, and the peptides extracted from *E. coli* average only 200 amino acids in length (Kiehn and Holland, 1970). Whether the purified polysomes are making abnormally long peptides, or whether a ribosome can translate more than one gene in a polygenic bacterial messenger without reinitiation, remains to be seen.

Acknowledgments

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