Specific polyadenylation and purification of total messenger RNA from *Escherichia coli*

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ABSTRACT

Obtaining pure mRNA preparations from prokaryotes has been difficult, if not impossible, for want of a poly(A) tail on these messages. We have used poly(A) polymerase from yeast to effect specific polyadenylation of *Escherichia coli* polysomal mRNA in the presence of magnesium and manganese. The polyadenylated total mRNA, which could be subsequently purified by binding to and elution from oligo(dT) beads, had a size range of 0.4–4.0 kb. We have used hybridization to a specific plasmid-encoded gene to further confirm that the polyadenylated species represented mRNA. Withdrawal of Mg^{2+} from the polyadenylation reaction resulted in addition of poly(A) to 16S rRNA despite the presence of Mn^{2+}, indicating the vital role of Mg^{2+} in maintaining the native structure of polysomes. Complete dissociation of polysomes into ribosomal subunits resulted in quantitative polyadenylation of both 16S and 23S rRNA species. Chromosomal *lacZ* gene-derived messages were quantitatively recovered in the oligo(dT)-bound fraction, as demonstrated by RT–PCR analysis. Potential advantages that accrue from the availability of pure total mRNA from prokaryotes is discussed.

INTRODUCTION

The large size of the eukaryotic genome, a very small proportion (1–2%) of which represents the coding sequences, as well as the organization of genes into exons and introns was the major impetus for the development of techniques for isolating pure mRNA from these organisms. The polyadenylated nature of eukaryotic mRNA was the single dominant factor contributing to the success of these techniques. The absence of significant polyadenylation in the messages of prokaryotes (1,2) precludes the application of such approaches for these organisms. This has, however, not been a major impediment owing to the vastly smaller sizes of prokaryotic genomes, almost all of which is represented by coding sequences. The advent of PCR has spawned several valuable techniques, such as subtractive hybridization and differential display of cDNAs, which have been applied to studies on regulation of gene expression in multiple eukaryotic and prokaryotic systems. The success of these highly demanding techniques is to a large extent dependent on the availability of pure mRNA devoid of the vast excess of rRNA present within

cells. Additionally, the absence of a post-transcriptionally added poly(A) stretch in prokaryotes in combination with the inherent instability of the prokaryotic message makes it extremely difficult to determine the authentic 3′-end of the mRNA and has consequently hampered studies on transcription termination in these organisms.

As a means to obtain total mRNA of reasonable purity from prokaryotes, we attempted specific polyadenylation of prokaryotic mRNAs. We hypothesized that in intact polysomes the 3′-ends of mRNAs alone, but not those of rRNAs, would be available for enzymatic manipulation. In the following report we show that treatment of intact polysomes with poly(A) polymerase leads to specific polyadenylation of mRNA. In support of our hypothesis, we also show that polyadenylation following dissociation of polysomes into ribosomal subunits results in effective polyadenylation of rRNA in addition to mRNA. The efficiency of polyadenylation of polysomal mRNA was further demonstrated by the quantitative recovery of IPTG-induced chromosomal *lac* operon-derived transcripts in the poly(A) fraction.

MATERIALS AND METHODS

Bacterial strains and plasmids

Escherichia coli BL21(DE3) was a kind gift of Dr William Studier (3). Plasmid pET3d-NS3(T) codes for a truncated 35.5 kDa protein from a 967 nt segment from the 5′-end of non-structural gene 3 (NS3) of the Indian isolate P20778 of Japanese encephalitis virus.

Isolation of polysomes

Escherichia coli BL21(DE3) with or without plasmid pET3d-NS3(T) was grown in Luria–Bertani broth to an $A_{600\ nm}$ of 0.6. Induction with 0.5 mM isopropyl β-D thiogalactopyranoside (IPTG) when required was for 2 h. Polysomes were isolated according to the method of Godson (4), with modifications. The cells were chilled rapidly in a mixture of ice and salt, centrifuged in a Sorvall GSA rotor at 7000 r.p.m. for 2 min and the supernatant drained thoroughly. The pellet was resuspended in ice-cold lysis buffer (10 mM Tris, pH 7.5, 1 mM $MgCl_2$, 0.1 mM ethylenediamine tetraacetic acid, 0.25 M potassium chloride, 0.1 mM dithiothreitol in 0.1 M sucrose), an equal weight of glass powder to that of the cell pellet was added and the cells ground on ice for 5 min in the presence of 1.0 mg/ml lysozyme and 100 µg/ml heparin. Deoxycholate and DNase I were then added to final concentrations of 1% and 50 µg/ml respectively and grinding was continued for a further 5 min. The lysate was centrifuged at 15 000 r.p.m. for

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10 min in a Sorvall SS34 rotor at 4°C and the clear supernatant (17 ml) layered on a sucrose step gradient consisting of 5.25 ml 2 M sucrose, 4.2 ml 1.5 M sucrose and 8.1 ml 0.5 M sucrose in lysis buffer. The gradient was centrifuged in a Beckman SW 28 rotor for 16 h at 27 000 r.p.m. at 4°C. Analysis of the quality of the polysomes was carried out by centrifuging part of the initial polysome pellet dissolved in lysis buffer through a 10–40% continuous sucrose gradient in a Beckman SW28 rotor for 2 h at 24 000 r.p.m. Fractions of 1 ml were collected and absorbance at 260 nm was determined.

Polyadenylation of polysomal mRNA

Aliquots of 1.5 $A_{260\,nm}$ units of polysomes were dissolved in 150 µl poly(A) polymerase buffer (20 mM Tris, pH 7.0, 60 mM potassium chloride, 0.7 mM manganese chloride, 3.5 mM magnesium chloride, 0.2 mM ethylenediamine tetraacetic acid, 0.6 mM dithiothreitol, 10% glycerol, 1 mM ATP) containing 1 µl (30 U) RNasin (Promega Corp.). To this was added 500 U (1 µl) yeast poly(A) polymerase (US Biochemicals) and the reaction was incubated at 30°C for 2 h. [α-^{32}P]ATP when used was adjusted to a final specific activity of 100 c.p.m./pmol.

In experiments where it was required to maintain the ribosomes in manganese alone as the sole divalent cation dissolution of the washed polysome pellet as well as the subsequent polyadenylation reaction were carried out in poly(A) polymerase buffer devoid of magnesium. To acheive complete dissociation of polysomes into ribosomal subunits, buffer lacking both magnesium and manganese was used. Tetracycline when used was at 100 µM.

RNA isolation and Northern analysis

Total RNA from the polysome preparation was isolated as follows. To the polysomes suspended in either lysis buffer or poly(A) polymerase buffer was added an equal volume of a solution containing 0.2 M Tris, pH 8.0, 2% SDS, 12% sodium *p*-amino-salicylate and 10 mM EDTA. The solution was then extracted with a 1:1 mixture of phenol equilibrated with 10 mM sodium acetate, pH 6.0, containing 0.1 M NaCl, 1 mM EDTA (5) and chloroform. RNA from the aqueous phase was precipitated by addition of a 1/10th vol. of 3 M sodium acetate, pH 6.0, followed by an equal volume of isopropanol. RNA from the Oligotex flow-through was extracted with phenol/chloroform and precipitated with isopropanol.

RNA samples were electrophoresed through 0.66 M formaldehyde–agarose gels in MOPS running buffer (50 mM MOPS, 10 mM sodium acetate, 2 mM EDTA, pH 7.0), transferred to nylon membrane and crosslinked with UV light. Hybridization to ^{32}P-labelled probes prepared by nick translation was carried out for 14 h at 68°C followed by extensive washing in 0.1× SSC at 68°C. Hybridization to end-labelled oligo(dT) (12–17mer) was carried out for 14 h at room temperature followed by washing in 3× SSC at 28°C. Probes were removed from the blots according to Sambrook *et al.* (6). Gels containing ^{32}P-labelled samples were dried between folds of filter paper and exposed to X-ray film.

Purification of poly(A) RNA

Oligotex mini kit from Qiagen GmbH was used according to the manufacturer's instructions. Briefly, 100 µl reaction mix was treated with 320 µl lysis buffer. The lysate was diluted with 840 µl dilution buffer, centrifuged at 12 000 *g* for 10 min to remove insoluble matter and 10 µl Oligotex beads were added to the clear supernatant. After 10 min at room temperature the beads were collected by centrifugation. The unbound fraction was retained for analysis. Beads were washed thrice with wash buffer and the bound poly(A) RNA, eluted with 100 µl 5 mM Tris, pH 7.5, at 70°C, was precipitated from 0.3 M sodium acetate and an equal volume of isopropanol.

RT-PCR and Southern analysis

Reverse transcription of the Oligotex flow-through and eluate fractions followed by PCR amplification of a 430 nt stretch from the *lacZ* gene was carried out using the Access RT-PCR kit from Promega Corporation according to the manufacturer's instructions. The sequences of the 5′ and 3′ primers were 5′-CGGCGGGCCAT-TACCAGGCC and 5′-AAGTCGCCGCGCCACTGGTG respectively. Reverse transcription was primed using the 3′ downstream primer mentioned above and RNA obtained from 0.5 $A_{260\,nm}$ units polysomes/reaction. PCR products were electrophoresed on a 1.2% agarose gel, transferred to nylon membranes and hybridized to *E.coli lacZ* sequences labelled using the Gene Images-CDP detection kit from Amersham Life Science.

RESULTS

Isolation and polyadenylation of polysomes

We achieved disruption of the *E.coli* cells by grinding extensively with glass powder. Cell debris was removed by high speed centrifugation at 17 000 *g* for 10 min. Polysomes free of contaminating monosomes were obtained by centrifugation of the lysate through a sucrose step gradient. Analysis of the polysomes on a continuous sucrose gradient revealed that the predominant species were 4mer, 5mer and 6mer, as reported previously (4), with a very small proportion comprising monosomes (data not shown).

The standard polyadenylation reaction was carried out in the presence of both magnesium and manganese ions at concentrations of 3.5 and 0.7 mM respectively. Whereas the former is required to maintain integrity of the polysomes, the latter is required for maximum activity of poly(A) polmerase (7). In order to find out if polyadenylation of any RNA species occurred, we carried out polyadenylation in the presence of ^{32}P-labelled ATP. As seen in Figure 1B (lane 1), heterogeneous RNA species ranging in size from 0.4 to ~4.0 kb (based on the mobility of the tracking dyes and rRNA) were found to have incorporated the radiolabel, although no ethidium bromide staining material other than the rRNAs was visible (Fig. 1A, lane 1). All the labelled material bound to and eluted from oligo(dT) beads (Fig. 1B, lane 3), further confirming that this population of RNA was indeed polyadenylated. Despite the presence of abundant rRNA, this material was quantitatively recovered in the flow-through fraction (Fig. 1A, lane 2) and no label was found in that fraction (Fig. 1B, lane 2). These results show that the 3′-ends of the rRNAs within intact polysomes are not available for enzymatic polyadenylation. We inferred that the polyadenylated species was mRNA based on the size range of labelled RNA.

Northern blot analysis of RNA

In order to confirm that the oligo(dT) bead-bound RNA was mRNA, polyadenylation was carried out on polysomes isolated from *E.coli* BL21(DE3) containing plasmid pET3d-NS3(T),
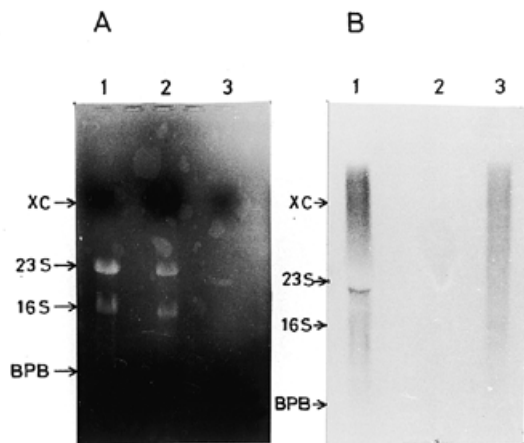
Figure 1. Polyadenylation of polysomes in the presence of α-^{32}P-labelled ATP. Polysomes were isolated from *E.coli* BL21(DE3) and polyadenylated under standard conditions of divalent cations in the presence of labelled ATP. (**A**) RNA samples electrophoresed on a 1% agarose gel and stained with ethidium bromide. (**B**) Autoradiogram of gel shown in (A). Lane 1, total RNA from polyadenylated polysomes; lane 2, oligo(dT) flow-through; lane 3, oligo(dT) bound fraction. XC and BPB refer to the positions of xylene cyanol and bromophenol blue respectively.

Figure 2. Polyadenylation of *E.coli* polysomes. Polysomes obtained from *E.coli* BL21(DE3) carrying the plasmid pET3d-NS3(T) were polyadenylated under standard conditions (3.5 mM Mg^{2+}, 0.7 mM Mn^{2+}) (**A**) RNA samples electrophoresed on a 1.5% agarose gel stained with ethidium bromide. (**B**) RNA samples shown in (A) transferred to nylon membrane and hybridized to ^{32}P-labelled NS3 sequences. (**C**) The nylon membrane shown in (B) was rehybridized to ^{32}P-labelled oligo(dT). Lane 1, total RNA from polysomes before polyadenylation; lane 2, total RNA from polysomes after polyadenylation; lane 3, oligo(dT) flow-through; lane 4, oligo(dT) bound fraction.

which carries a 967 nt segment of the NS3 gene of Japanese encephalitis virus. Lanes 1 and 2 of Figure 2A show total RNA obtained from polysomes before and after polyadenylation respectively. All the rRNA was again found in the flow-through fraction of oligo(dT) beads (Fig. 2A, lane 3). The various fractions were then hybridized to NS3 sequences labelled by nick translation. A signal around the expected size of 967 bp was obtained in the total RNA fractions (Fig. 2B, lanes 1 and 2), however, the size of the NS3-specific sequences had undergone an upward shift following polyadenylation (lane 2). This material efficiently bound to the oligo(dT) beads (lane 4). The presence of some NS3 hybridizing material also in the flow-through fraction (Fig. 2B, lane 3) indicates that ~50% of the NS3 mRNA was not polyadenylated. Rehybridization of the membrane shown in Figure 2B to end-labelled oligo(dT) further confirmed that addition of poly(A) was achieved by incubation with poly(A) polymerase (Fig. 2C, lane 2) and that the material which bound to and eluted from oligo(dT) beads was in fact polyadenylated (Fig. 2C, lane 4).

Requirement of Mg^{2+} for specific polyadenylation of mRNA

Yeast poly(A) polymerase has been reported to function with either divalent cation, magnesium or manganese, with a markedly higher activity in manganese (7). Magnesium is known to play a vital role in maintaining the association of ribosomal subunits in the 70S complex (8). However, among a number of divalent cations that have been tested for their ability to substitute for this role of magnesium, manganese has been reported to efficiently replace magnesium (9,10). We therefore wished to carry out polyadenylation of intact polysomes with manganese as the sole divalent cation. For this purpose the polysome pellet was dissolved in poly(A) polymerase buffer lacking Mg^{2+} but containing 0.7 mM Mn^{2+}. This manganese ion concentration is known to be sufficient to maintain the ribosome in the 70S complex, as judged by sedimentation through sucrose gradients (10). Polyadenylation of
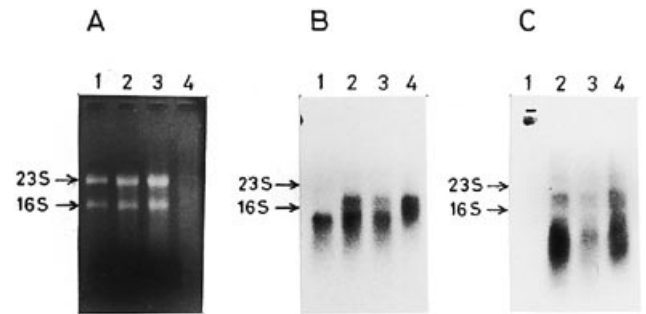
polysomes in the presence of manganese alone led to addition of poly(A) to 16S rRNA, resulting in an upward shift of this species (Fig. 3A, lane 2). To confirm that the increase in size of 16S rRNA was due to addition of poly(A), the RNA samples in Figure 3A were hybridized to end-labelled oligo(dT). Figure 3C shows specific hybridization of this probe to the size-shifted 16S rRNA in lane 2 but not in lane 1. In addition, this species of 16S rRNA bound quantitatively to oligo(dT) beads (Fig. 3A and C, lane 4). In order to determine if this observation was due to the absence of magnesium, we added magnesium at a final concentration of 3.5 mM to the reaction mix soon after addition of the enzyme to polysomes suspended in buffer containing only manganese. The presence of magnesium brought about a reduction in both the proportion and extent of size increase of 16S rRNA following polyadenylation (Fig. 3A, lane 5). As expected, the polyadenylated fraction of 16S rRNA bound to oligo(dT) beads (Fig. 3A and C, lane 7).

It has been shown that yeast poly(A) polymerase has highest affinity for the 3′-ends of rRNA among various RNA species tested (7). We therefore wished to find out if polyadenylation of 16S rRNA occurred at the expense of mRNA. Hybridization of the RNA samples in Figure 3A to labelled NS3 sequences clearly showed polyadenylation of this species (Fig. 3B, lanes 4 and 7). Polyadenylation of mRNA was also indicated by hybridization of labelled oligo(dT) to material ranging in size from 0.4 to 4.0 kb (Fig. 3C).

Polyadenylation of dissociated polysomes

Our hypothesis that polyadenylation of intact polysomes should result in specific poly(A) addition to mRNA was based on the assumption that in an intact polysome the 3′-ends of rRNAs would not be accessible to poly(A) polymerase. It would follow, therefore, that polyadenylation following complete dissociation of polysomes into ribosomal subunits should result in effective polyadenylation of both major rRNA species. We achieved dissociation of polysomes by excluding both magnesium and manganese from the buffer used to resuspend the polysome pellet. However, poly(A) polymerase has a requirement for either magnesium or manganese for activity. We therefore carried out
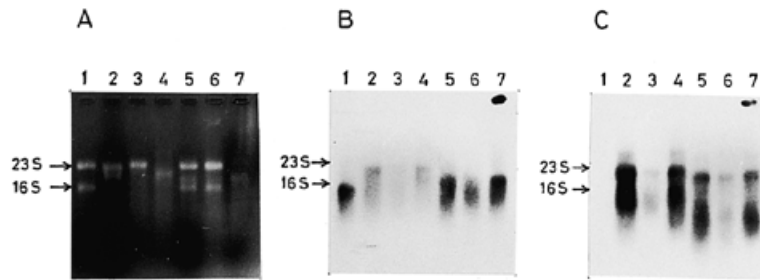
Figure 3. Requirement of Mg^{2+} for specific polyadenylation of mRNA. Polysomes were isolated from *E.coli* BL21(DE3) carrying pET3d-NS3(T). (**A**) RNA samples electrophoresed on a 1.5% agarose gel stained with ethidium bromide. (**B**) Samples shown in (A) transferred to nylon membrane and hybridized to ^{32}P-labelled NS3 sequences. (**C**) Nylon membrane shown in (B) rehybridized to ^{32}P-labelled oligo(dT). Lanes 1–4 represent samples from polysomes polyadenylated in the presence of 0.7 mM Mn^{2+} alone, while lanes 5–7 represent samples from polysomes dissolved initially in buffer containing 0.7 mM Mn^{2+} alone but polyadenylated after addition of Mg^{2+} as described in Results . Lane 1, total RNA from polysomes before polyadenylation; lanes 2 and 5, total RNA after polyadenylation; lanes 3 and 6, oligo(dT) flow-through; lanes 4 and 7, oligo(dT) bound fraction.
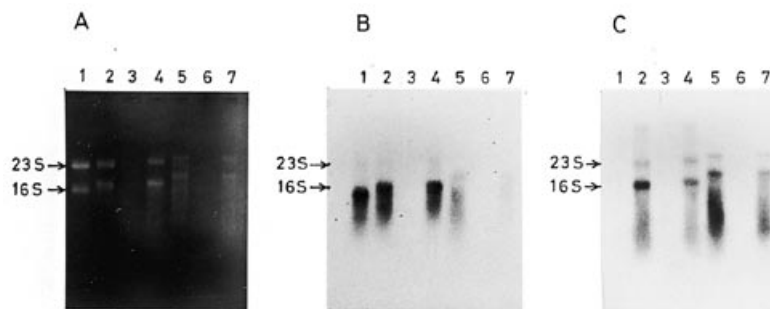

Figure 4. Polyadenylation of dissociated polysomes. Polysomes were isolated from *E.coli* BL21(DE3) carrying pET3d-NS3(T) and dissociated by suspending in buffer lacking divalent cations. (**A**), (**B**) and (**C**) are as in Figure 3. Lanes 1–4 represent samples obtained from polysomes polyadenylated in presence of 1 mM Mg^{2+}, while lanes 5–7 represent samples from polysomes polyadenylated in the presence of 100 µM tetracycline, 0.7 mM Mn^{2+} and 3.5 mM Mg^{2+}. Samples in lanes 1–7 are as in Figure 3.

polyadenylation of dissociated polysomes in the presence of 1 mM magnesium, a concentration less than the optimum 3.5 mM recommended for poly(A) polymerase. The reassociation kinetics of ribosomal subunits at this concentration of magnesium has been reported to be slow (10). Figure 4A, lane 2 revealed polyadenylation of both major rRNAs under these conditions, judged by an increase in size, and resulted in hybridization of these two species to labelled oligo(dT) (Fig. 4C, lane 2). Both these species also bound to oligo(dT) beads (Fig. 4A and C, lane 4). Under these conditions, however, slow re-association of ribosomal subunits during the polyadenylation reaction cannot be prevented. In order to maintain the ribosomal subunits in a dissociated state during the polyadenylation reaction we did the following: after dissolution of the polysomal pellet in buffer devoid of manganese and magnesium, tetracycline was added to a final concentration of 100 µM. Tetracycline is known to bind tightly to the 30S ribosomal subunit and prevent its re-association with the 50S subunit to form ribosomes (11). Polyadenylation of tetracycline-dissociated polysomes was carried out in standard polyadenylation buffer with magnesium (3.5 mM) and manganese (0.7 mM). Lane 5 of Figure 4A revealed an enhanced upward size shift of the rRNAs under these conditions as compared with that seen in the presence of 1 mM magnesium (Fig. 4A, lane 2). This may be due either to maximum accessibility of the 3′-ends of the rRNAs upon total dissociation or due to enhanced activity of the enzyme in the presence of optimal concentrations of both divalent cations. Hybridization to labelled oligo(dT) confirmed that the size increase

was due to addition of a stretch of A residues (Fig. 4C, lanes 5 and 7). Hybridization to NS3 sequences once again proved that messages were also polyadenylated under these conditions (Fig. 4B). Obviously, tetracycline at this concentration did not inhibit the activity of poly(A) polymerase.

RT-PCR and Southern analysis of RNA fractions

Having defined conditions required for achieving specific polyadenylation of mRNA, we next set out to show that a chromosomally derived message transcribed upon induction from a single copy gene, as opposed to an abundant plasmid-encoded transcript, could be identified solely in the polyadenylated fraction. Polysomes prepared from *E.coli* BL21(DE3) grown in Luria–Bertani broth and subjected to IPTG induction for 2 h were polyadenylated and the poly(A)-containing species recovered. Reverse transcription of this as well as the oligo(dT) flow-through fraction was carried out using a 20mer oligonucleotide mapping in the *lacZ* gene of the *lac* operon, followed by PCR amplification using a second primer 430 nt upstream. Figure 5A shows the ethidium stained agarose gel following electrophoresis of the PCR products. The expected 430 nt species was generated only from the polyadenylated fraction (lane 2), identical to the product derived from genomic DNA of *E.coli* BL21(DE3) (lane 1), and was totally absent in the oligo(dT) flow-through material (lane 3). This PCR product also specifically hybridized to labelled *lacZ* sequences (Fig. 5B, lanes 1 and 2). These results indicate that
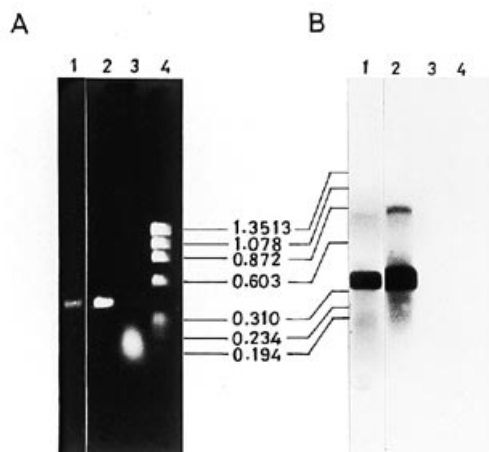
Figure 5. RT-PCR and Southern analysis of *lacZ* mRNA. (**A**) PCR products were electrophoresed on a 1.2% agarose gel and stained with ethidium bromide. (**B**) Samples shown in (A) were transferred to nylon membrane and hybridized to a 3.0 kb *lacZ* gene labelled using the Gene Images kit from Amersham. Lane 1, genomic DNA from *E.coli* BL21(DE3); lane 2, oligo(dT) eluate; lane 3, oligo(dT) flow-through; lane 4, *Hae*III-digested φX DNA. Sizes are given in kilobases.

in contrast to overexpressed plasmid-derived messages, bacterial chromosome-encoded mRNA species can be quantitatively polyadenylated and recovered following polyadenylation of purified polysomes.

DISCUSSION

We have achieved specific polyadenylation of mRNA from *E.coli* by the action of poly(A) polymerase on polysomes. In keeping with our hypothesis, our results clearly revealed that in intact polysomes the 3′-ends of mRNA only, but not rRNA or 5S RNA, are available for enzymatic addition of poly(A). As further proof of this hypothesis, total dissociation of polysomes into ribosomal subunits facilitated quantitative poly(A) addition to 16S and 23S rRNAs. Yeast poly(A) polymerase shows a 10-fold increased activity in the presence of Mn^{2+} as compared with Mg^{2+}. However, we retained Mg^{2+} at 3.5 mM in order to maintain the integrity of polysomes. Under the standard reaction conditions ~100–200 A residues appeared to be added based on the size increase observed in agarose gels. Use of *E.coli* BL21(DE3) carrying the NS3 gene of a flavivirus driven by the T7 promoter facilitated monitoring of polyadenylation of a single abundant species of mRNA. However, in spite of carrying out the polyadenylation reaction for 2 h, only 50% of the NS3 messages were polyadenylated. This suggests that not all polysomes make the 3′-ends of their messages available for poly(A) addition. In prokaryotic cells, where transcription is coupled to translation, these polysomes may represent the fraction where transcription is still in progress. If this were so, this effect is unlikely to alter the representation of any specific message in the polyadenylated fraction. However, this phenomenon was pronounced only in the case of abundantly transcribed messages, since the chromosomal *lacZ* messages were quantitatively present in the polyadenylated fraction.

Polyadenylation in the presence of manganese alone led to addition of poly(A) to 16S rRNA in addition to mRNA. This result was unexpected in view of the reported ability of manganese at the concentration used to substitute for magnesium in maintaining the association of ribosomal subunits (10). However, this result is in accordance with the following earlier observations: (i) ribosomes prepared with manganese, although exhibiting normal sedimentation values, retained only 10% of their amino acid polymerizing activity (12); (ii) manganese-bound ribosomal subunits showed altered ribonuclease sensitivity (9); (iii) ribosomal subunits unfolded by depletion of divalent cations can be refolded by providing manganese, however, these have an altered conformation revealed by altered viscosity (13). The foregoing results indicate that ribosomes in the presence of manganese alone are structurally and functionally different from authentic magnesium-bound ribosomes. We therefore concluded that these changes may be responsible for the availability of 16S rRNA for polyadenylation in manganese-bound ribosomes. The preferential polyadenylation of 16S rRNA in the presence of manganese alone also suggested that 30S subunits are more susceptible to alteration under these conditions. In support of this conclusion, changes in sensitivity to RNase A in the presence of manganese have also been reported to be more pronounced in the 30S than in the 50S subunit (9). These changes may involve either the RNA or the proteins or both in the 30S subunit. In addition, several cations in the absence of magnesium were reported to bring about 'reciprocal structural rearrangements' confined predominantly to the decoding region (around positions 1400–1500) which maps to the 3′-end of 16S rRNA (14). It is therefore likely that our observations were due to structural rearrangements in the 16S rRNA.

The above effect observed in manganese-bound ribosomes could be potentially reversed by the addition of magnesium to the polyadenylation reaction. In keeping with this observation, Tissieres *et al.* (12) have reported restoration of amino acid polymerizing activity of manganese-bound ribosomes by addition of magnesium. However, a fraction of the 16S rRNA was still observed to be polyadenylated. Presumably restoration of the polysomes to their native state occurred over a period of time during polyadenylation, contributing to the partial nature of the reaction.

The ability to obtain pure mRNA devoid of rRNA from prokaryotes has several applications. It now becomes feasible to construct cDNA libraries for prokaryotic organisms. Bacteria are known to regulate expression of their genes in response to their environment. Examples include genes encoding enzymes required for utilization of nutrients, sporulation-specific proteins and virulence factors of pathogenic bacteria that are synthesized in response to the host environment. Techniques such as subtractive hybridization (15) and DDRT-PCR (16,17) have been widely exploited to identify such genes. Whereas the use of pure mRNA in the former technique can result in fewer false positives that may arise from the vast excess of rRNA, its use in the latter is bound to increase the probability of identifying candidate genes. Indeed, we have demonstrated that messages transcribed from an induced operon such as *lacZ* can be identified by RT-PCR analysis of the poly(A)-containing fraction. In addition, the attachment of a poly(A) stretch *in vitro* to authentic 3′-terminii of RNA may facilitate studies on transcription termination in prokaryotes.

Several studies have addressed the question of *in vivo* addition of poly(A) to mRNA in prokaryotes. Perry *et al.* (1) reported the complete absence of polyadenylation of messages in *E.coli*. However, studies by Nakazato *et al.* (2) revealed limited polyadenylation of *E.coli* mRNA; as little as 3% of mRNA

molecules were found to contain poly(A) sequences long enough (25–45 nt) to bind to oligo(dT)–cellulose. Quantitative recovery of the mRNA population therefore necessitates polyadenylation *in vitro*.

ACKNOWLEDGEMENTS

REFERENCES

1 Perry,R.P., Kelley,D.E. and La Torre,J. (1972) *Biochem. Biophys. Res. Commun.*, **48**, 1593–1600.
2 Nakazato,H., Venkatesan,S. and Edmonds,M. (1975) *Nature*, **256**, 144–146.
3 Studier,F.W., Rosenberg,A.H., Dunn,J.J. and Dubendorff,J.W. (1990) *Methods Enzymol.*, **185**, 60–89.
4 Godson,G.N. (1967*) Methods Enzymol.*, **XIIA**, 503–517.
5 Perry,R.P., La Torre,J., Kelley,D.E. and Greenberg,J.R., (1972) *Biochim. Biophys. Acta*, **262**, 220–226.
6 Sambrook,J., Fritsch,E.F. and Maniatis,T. (1989) *Molecular Cloning: A Laboratory Manual*, 2nd Edn. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY.
7 Lingner,J., Radtke,I., Wahle,E. and Keller,W. (1991) *J. Biol. Chem.*, **266**, 8741–8746.
8 Weiss,R.L. and Morris,D.R. (1973) *Biochemistry*, **12**, 435–441.
9 Weiss,R.L., Kimers,B.W. and Morris,D.R. (1973) *Biochemistry*, **12**, 450–456.
10 Roskoski,R.,Jr (1969) *Arch. Biochem. Biophys.*, **130**, 561–566.
11 Jay,G. and Kaempfer,R. (1975) *J. Biol. Chem.*, **250**, 5742–5748.
12 Tissieres,A., Schlessinger,D. and Gros,F. (1960) *Proc. Natl. Acad. Sci. USA*, **46**, 1450–1463.
13 Tal,M. (1969) *Biochim. Biophys. Acta*, **195**, 76–86.
14 Moazed,D., Vanstolk,B,J., Douthwaite,S. and Noller,H.F. (1986). *J. Mol. Biol.*, **191**, 483–493.
15 Lee,S.W., Tomasetto,C. and Sager,R. (1991) *Proc. Natl. Acad. Sci. USA*, **88**, 2825–2829.
16 Liang,P. and Pardee,A.B. (1992) *Science*, **257**, 967–971.
17 Kwaik,Y.A. and Pederson,L.L. (1996) *Mol. Microbiol.*, **21**, 543–556.