Identification of the coding region for a second poly(A) polymerase in Escherichia coli

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ABSTRACT We had earlier identified the pcnB locus as the gene for the major Escherichia coli poly(A) polymerase (PAP I). In this report, we describe the disruption and identification of a candidate gene for a second poly(A) polymerase (PAP II) by an experimental strategy which was based on the assumption that the viability of E. coli depends on the presence of either PAP I or PAP II. The coding region thus identified is the open reading frame f310, located at about 87 min on the E. coli chromosome. The following lines of evidence support f310 as the gene for PAP II: (i) the deduced peptide encoded by f310 has a molecular weight of 36,300, similar to the molecular weight of 35,000 estimated by gel filtration of PAP II; (ii) the deduced f310 product is a relatively hydrophobic polypeptide with a pl of 9.4, consistent with the properties of partially purified PAP II; (iii) overexpression of f310 leads to the formation of inclusion bodies whose solubilization and renaturation yields poly(A) polymerase activity that corresponds to a 35-kDa protein as shown by enzyme blotting; and (iv) expression of a f310 fusion construct with hexahistidine at the N-terminus of the coding region allowed purification of a poly(A) polymerase fraction whose major component is a 36-kDa protein. E. coli PAP II has no significant sequence homology either to PAP I or to the viral and eukaryotic poly(A) polymerases, suggesting that the bacterial poly(A) polymerases have evolved independently. An interesting feature of the PAP II sequence is the presence of sets of two paired cysteine and histidine residues that resemble the RNA binding motifs seen in some other proteins.

The recent identification of the pcnB locus (1) as the gene for the major poly(A) polymerase (ATP:polynucleotide adenyllyltransferase, EC 2.7.7.19) of Escherichia coli (2) has opened the door to the analysis of the function and mechanism of RNA polyadenylation in prokaryotes on the molecular level. However, although it is clear that the poly(A) polymerase (PAP I) encoded by pcnB is essential for the 3'-adenylation of the antisense RNAs that control the replication of certain plasmids (3–6), the observation that disruption of the pcnB locus by a mini-kan insertion (7) leads only to a modest reduction in the level of mRNA polyadenylation (2) suggests that E. coli has more than one poly(A) polymerase. Indeed, we were able to identify a second poly(A) polymerase (PAP II) in extracts of E. coli with a pcnB deletion (8).

To elucidate the function of polyadenylation of mRNA in E. coli, it is essential to define the relative roles of the two poly(A) polymerases in RNA metabolism and processing. This can be accomplished only if the genes for both enzymes are identified. In this paper, we describe our successful strategy for disrupting the gene for PAP II in the absence of a functional chromosomal gene for PAP I, which has led to the identification of the previously unassigned open reading frame f310 (9) as the gene encoding PAP II.

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MATERIALS AND METHODS

Bacterial Strains and Plasmids. The E. coli strains used in this study were SK6591 (thyA715, pnp7) (10), SK6699 (thyA, pnp7, pcnB::kan) (8), E. coli MZ1 [his ilv rpsL, galKam pgllΔ8(bio-uvrB)ΔH1α'S (c1857 N res)] (11). E. coli InvAα' [endA1 recA1 hsdR17 (r59 m12) supE44 λ- thi-1 gyrA96 relA1 F80 lacZΔM15 Δ(lacZYA-argF)U169] (Invitrogen) and E. coli BL21 (DE3) [F- ompT (f81m9) DE3] (12) Strains were grown in Luria–Bertani medium and antibiotics were used at the following concentrations: 50 μg/ml ampicillin, 50 μg/ml kanamycin, and 10 μg/ml chloramphenicol.

Plasmid vector pBAD18 (13), containing the araBAD promoter and the regulatory gene araC, was the gift of Luz-Maria Guzman (Harvard Medical School). pRE1 (14), a vector designed for the controlled expression of lethal genes, was the gift of P. Reddy (National Institute of Standards and Technology). pRE1-pcnB (designated pRE1-1 in ref. 2), a pRE1 derivative containing the entire pcnB coding region, was described earlier (2). pCRII version 2.2. a vector for the direct cloning of PCR products, was from Invitrogen. PET28a, a vector for the N-terminal fusion of a gene product to hexahistidine and expression from a T7/lac promoter, was from Novagen. ANK1324 (15), a vehicle for the delivery of a mini-Tn10 carrying the cat gene of pACYC184, was obtained from Jon Beckwith (Harvard Medical School).

Materials. Restriction endonucleases were from New England BioLabs; Taq DNA polymerase from Perkin–Elmer/ Cetus; L-arabinose from Flansteilh Laboratories; His-bind metal chelation resin from Novagen; [3H]ATP from Moravek Biochemicals (Brea, CA); and poly(A) from Pharmacia. Oligonucleotides were synthesized by the phosphoramidite method with a MilliGen Expedite DNA synthesizer.

Poly(A) Polymerase Assay. Poly(A) polymerase was assayed as described (8). Reaction volumes of 50 μl were used, containing 50 mM Tris-HCl (pH 8.0), 10 mM MgCl2, 2 mM MnCl2, 1 mM EDTA, 1 mM DTT, 100 mM NaCl, 80 mM KCl, 5 μM rifampicin, 1 mM phosphoenol pyruvate, 3 μg of pyruvate kinase, 25 μg of gelatin, 0.12 mM [3H]ATP (50 μCi/μmol; 1 Ci = 37 GBq), and 10 μg poly(A) primer. After 10 min at 37°C, poly(A) was adsorbed onto DE-52 filter discs and radioactivity was determined in a liquid scintillation spectrometer.

Enzyme Blot Analysis. E. coli MZ1 transformed with pRE1, pRE1-f310, or pRE1-pcnB were grown in medium E (16) supplemented with 0.8% Difco nutrient broth, 0.5% glucose, and 25 μg/ml ampicillin at 30°C to A600 of 0.55, and were induced for 1 h at 39°C. Cells were disrupted in a French pressure cell, and aliquots of the crude extract (3 μl), together with a mixture of prestained proteins of known molecular weight (Bio-Rad), were subjected to SDS/PAGE in 12.5% polyacrylamide gels, followed by electroblotting onto Problot membrane (Applied Biosystems) in 0.1 M 3-(cyclohexylamino)-propanesulfonate buffer (pH 11.0) containing 20% methanol. The membrane was screened for poly(A) polymerase activity

Abbreviations: PAP I and II, poly(A) polymerase I and II; IPTG, isopropyl β-D-thiogalactoside.
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by incubation for 16 h at 23°C in 7 ml of a solution containing 50 mM Tris-HCl (pH 8.0), 180 mM NaCl, 10 mM MgCl₂, 2 mM MnCl₂, 2 mM EDTA, 1 mM DTT, 160 µg/ml streptolydigin, 10 mM phosphocreatine, 100 µg/ml creatine kinase, 0.12 mM [α-35S]ATP (100 µCi/µmol), and 70 µg poly(A) primer. The membrane was then washed at 4°C twice with 1.2 M trichloroacetic acid containing 2.5 mM ATP, four times with 0.6 M trichloroacetic acid, and once with water, dried at room temperature, and subjected to autoradiography using a storage phosphor screen and analyzed on a Molecular Dynamics PhosphorImager SF with IMAGEQUANT software.

Isolation and Renaturation of PAP II from Inclusion Bodies. E. coli MZ1 harboring either pRE1 or pRE1-f310 were grown in medium E (16) supplemented with 0.8% Difco nutrient broth, 0.5% glucose, and 25 µg/ml ampicillin at 30°C to OD₅₆₀ of 0.55, and were induced for 1 h at 39°C. Cells from 250 ml culture were harvested and disrupted in 2 ml of buffer E (50 mM Tris-HCl, pH 7.9/200 mM KC1/10 mM MgCl₂/1 mM DTT/1 mM EDTA/5% glycerol/0.5 mM phenylmethylsulfonyl fluoride) in a French Pressure cell. Inclusion bodies, together with other particulate material, were sedimented by centrifuging the crude cell lysate at 20,000 × g for 15 min. Pellets were washed twice with buffer E by dispersing and recentrifuging the suspension. The washed pellet was extracted by homogenizing manually every 10 min with 0.5 ml of denaturation buffer, 6 M urea containing 20 mM Tris-HCl (pH 8.5), 2 mM EDTA, and 10 mM DTT for 1 h at 4°C. The suspension was centrifuged and the solubilized proteins were renatured as described by Nagai et al. (17) by dialysis against 100 ml of the first dialysis buffer (2 M urea/20 mM Tris-HCl pH 8.0/200 mM KC1/5 mM DTT/1 mM EDTA) for 14 h at 4°C and then against 200 ml of the second dialysis buffer (20 mM Tris-HCl pH 8.0/200 mM KC1/5 mM DTT/1 mM EDTA/10% glycerol) for 8 h.

Purification and Renaturation of N-Terminal Hexahistidine-Tagged PAP II from Inclusion Bodies. E. coli strain BL21(DE3), transformed with pET28a or pET28a-f310, was grown in Luria–Bertani medium plus kanamycin (30 µg/ml) to , then induced for 3 h by the addition of 1 mM isopropyl β-D-thiogalactoside (IPTG). Harvested cells (110 mg wet weight) were resuspended in 2 ml of buffer B (20 mM Tris-HCl, pH 7.9/500 mM NaCl/5 mM imidazole) and cells were disrupted in a French pressure cell. Inclusion bodies were collected by centrifugation at 20,000 × g for 15 min and dispersed in 0.25 ml of buffer B containing 6 M urea. After 1 h at 4°C to allow for solubilization, the hexahistidine-tagged protein was purified by batch-mode metal-ion-affinity chromatography. The sample was absorbed onto 0.2 ml of Ni²⁺-Sepharose (Novagen) in buffer B with 6 M urea. The resin was spun down and washed twice with 1 ml of buffer B with 6 M urea and twice with 1 ml of binding buffer B with 6 M urea and 20 mM imidazole. Bound proteins were then eluted by 0.4 ml of buffer B with 6 M urea and 300 mM imidazole. The eluted protein was renatured by the two-step dialysis procedure of Nagai et al. (17) as described above.

Other Methods. DNA amplification by PCR and DNA sequencing were carried out as described (2).

RESULTS

Strategy for Identifying the Gene for a Second Poly(A) Polymerase. Our experimental approach was predicated on the assumption that the absence of both PAP I and PAP II is lethal to E. coli. Accordingly, we developed the following four-step strategy for identifying the PAP II gene: (i) deletion of the chromosomal penB locus and introduction of a plasmid copy of penB under control of the arabinose promoter; (ii) transposon mutagenesis and isolation of arabinose-dependent transposon mutants; (iii) amplification of the transposon-disrupted locus by inverse PCR, followed by cloning, sequencing, and comparison to the E. coli genome data base; and (iv) overexpression of the gene thus identified and assay for poly(A) polymerase activity.

Construction of an E. coli Strain with penB Under Arabinose Control. The starting point for our construction was E. coli strain SK6699 (8), which lacks the gene for polynucleotide phosphorylase (pnp) and has a disrupted PAP I locus (pnpB::kan). The aim was to introduce into this host a plasmid vector carrying an arabinose-inducible penB gene. The vector of choice was pBAD18 (13), which confers ampicillin-resistance and contains the araBAD promoter (pBAD) and arac, a tight regulator of pBAD which allows expression only in the presence of arabinose. To insert penB adjacent to the pBAD promoter, we used PCR to amplify this gene from plasmid pREI-1 (2), using primers chosen so as to allow amplification of the complete coding sequence for PAP I. The PCR product was inserted into pBAD18 to yield pBAD18–penB. When E. coli SK6699, which had only 20% of the normal poly(A) polymerase levels of the parent strain (E. coli SK5691), was transformed with pBAD18–penB, poly(A) polymerase levels increased 35-fold upon induction with arabinose (data not shown).

Transposon Mutagenesis of E. coli SK6699/pBAD18–PAPI. To disrupt the gene for PAP II in the E. coli strain SK6699/ pBAD18–penB, we used the minitransposon Tn10 vehicle, ANK1324 (15), and selected chloramphenicol-resistant colonies by plating phage-infected cells on agar containing ampicillin (to maintain pBAD/PAP), chloramphenicol, and arabinose. Ten thousand colonies were then screened for growth on two kinds of plates containing Luria–Bertani agar with both antibiotics and either 0.02% arabinose or none. A single ampicillin- and chloramphenicol-resistant colony, T99, was found which grew only in the presence of arabinose but not in its absence, the anticipated phenotype of a strain with minicam disruption of PAP II gene. The phenotype of colony T99 was confirmed by streaking on Luria–Bertani agar plates with the two antibiotics and with or without 0.02% arabinose, but all subsequent efforts to subculture this strain, either on plates or in liquid media, were unsuccessful. In hindsight, such loss of viability should have been expected, because arabinose, at the low level (200 µg/ml) used in the agar, would have been substantially depleted around the bacterial colonies, curtailing the synthesis of PAP I. This could have been avoided by subculturing before the colonies had reached their maximum size, as was done in the original screening.

Amplification and Identification of the Transposon-Disrupted Gene. The transposon-disrupted gene from the nonviable arabinose-inducible strain T99 colony was recovered by inverse PCR. Chromosomal DNA (5 µg) from cells of colony T99 scraped from the agar plate was partially digested with Sau3A I, followed by religation at a relatively low DNA concentration (1.25 ng/ml) to encourage circularization. The circular DNA was then amplified by inverse PCR, using primers complementary to the 70-bp inverted repeats at the ends of mini-Tn10 (cat), which conveniently contain Sau3A I sites outside the primer sequence selected (15), as described by Higashitani et al. (18). The inverse PCR products were ligated to vector pCRII and used to transform E. coli InvacFᵀ. Recombinant clones with inserts were identified and the nucleotide sequence of the chromosomal regions adjacent to the inverted repeats at the end of Tn10/insert of two of the clones, T4 and T8, were determined.

Sequences of 50 and 94 bp were obtained from clones T4 and T8, respectively, and used to search for homologous segments in the E. coli DNA data base. Both sequences found exact matches in a region near 87 min on the E. coli DNA data base, sequenced earlier by Blattner and coworkers (9). As shown in Fig. 1, the two matches were in the same open reading frame termed f310, which had not yet been assigned a function and whose deduced product is a 310-residue protein of Mr 36,300, in reasonable agreement with the estimated Mr of 35,000 for the second poly(A) polymerase of E. coli (8).
Expression of Poly(A) Polymerase Activity from the Cloned f310 Gene. To determine whether f310 indeed encodes a poly(A) polymerase, we amplified the f310 gene from E. coli chromosomal DNA by PCR using mismatched primers to generate an Ndel site near the ATG initiation codon and a BamHI site after the TAA stop codon. The amplified fragment was ligated into the pRE1 expression vector, and the recombinant plasmid, pRE1-f310, was used to transform E. coli MZ21. The transformed strain, as well as a control strain with nonrecombinant pRE1, were grown at 30°C and αPL expression was induced by temperature shift to 39°C for 1 h. Extracts from induced and uninduced cultures from the two strains were prepared by disruption with a French pressure cell and assayed for poly(A) polymerase activity either directly or after centrifugation at 100,000 × g. No differences in poly(A) polymerase activity were seen in the extracts from cells transformed with either pRE1-f310 or the control plasmid pRE1 (data not shown). Analysis of the same extracts by SDS/PAGE showed an overexpressed 36-kDa protein in the strain transformed with pRE1-f310, which was completely sedimented at 100,000 × g, presumably as inclusion bodies (see Fig. 34). To determine whether poly(A) polymerase activity was associated with the overexpressed 36-kDa protein, we used a blotting-enzyme procedure.
PAP I for polymerase activity

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FIG. 2. SDS/PAGE and enzyme blot analysis of proteins produced by E. coli strains transformed with pREI carrying the f310 gene. The transformed cells were grown and disrupted and the cell extracts were processed as described. SDS/PAGE was carried out in 12.5% polyacrylamide gels together with a mixture of proteins of known molecular weight, followed by staining with Coomassie blue. The overexpressed 36-kDa protein is indicated by arrows. (A) Crude cell extracts (10 μg of protein) from E. coli MZ1 transformed with pREI or pRE1-f310 and induced for 1 h at 39°C. (B) Crude cell extracts (3 μl) from E. coli MZ1 transformed with pREI, pRE1-f310, or pRE1-pcnB and induced for 1 h at 39°C were subjected to SDS/PAGE as in A and then blotted onto a Problot membrane and assayed for poly(A) polymerase activity as described. pRE1-pcnB, which carries the gene for PAP I (2), was included as a positive control.

renatured extracts, with similar extracts from E. coli MZ1 containing pREI vector alone as a control. Renatured extracts from the insoluble fraction of E. coli MZ1/pRE1-f310 had an activity of 2 units/mg protein, whereas an analogous preparation from the host strain transformed with the control vector had one-fifteenth as much activity (Fig. 3A). However, SDS/PAGE showed that the solubilized material from cells with pRE1-f310 was highly heterogeneous (data not shown).

Purification and Renaturation of Overexpressed PAP II as a Hexahistidine Fusion Protein. To ascertain that the regenerated activity is due to the overexpression of a single polypeptide encoded by the f310 gene, we recloned the f310 gene sequence into expression vector pET28a so as to fuse six consecutive histidine residues to the N terminus of the expressed protein and allow its purification by chelation to a Ni²⁺ column (19). The f310 sequence was excised from plasmid pRE1-f310 with NdeI and BamHI and ligated to vector pET-28a cut with the same restriction enzymes, and the recombinant plasmid, pET-28a-f310, was used to transform E. coli BL21 (DE3). A parallel control experiment was done with E. coli BL21 (DE3) transformed with nonrecombinant pET28a. Expression was induced by adding 1 mM IPTG to exponentially growing cultures of the transformed strain. Examination of the extracts of the induced cells by SDS/PAGE revealed the presence of overexpressed 36-kDa protein in the insoluble fraction but not in the supernatant, consistent with the formation of inclusion bodies (Fig. 4A). Exploiting the His-Tag sequence at the N terminus of the overexpressed protein, we purified the overexpressed protein from the insoluble fraction by solubilization in 6 M urea, followed by affinity chromatography as described in Materials and Methods. As shown in Fig. 4B, the polypeptides which eluted from the Ni²⁺ column with 1 M imidazole buffer consisted of single major 36-kDa band, together with two minor bands of higher molecular weight. In view of the fact that PAP II contains nine cysteine residues and purification had to be carried out in thiol-free buffers, it is possible that the latter may represent disulfide-linked multimers of the fusion protein or co-aggregates with other proteins (20). A 36-kDa component could not be recognized in the corresponding fractions from cells transformed with nonrecombinant pET28a. The purified polypeptide fractions from cells transformed with pET28a-f310 as well as from control cells were renatured by a two-stage dialysis procedure (17) and poly(A)-polymerase activity was measured. Substantial poly(A) polymerase activity was found in the purified protein fraction from cells transformed with the vector carrying the f310 gene but not in the corresponding fraction from control cells (Fig. 3B), suggesting that the f310 gene encodes a polypeptide with poly(A) polymerase activity.

FIG. 3. Assay of poly(A) polymerase activity in extracts of E. coli strains transformed with expression vectors carrying the f310 gene. (A) The insoluble fraction from induced cells of E. coli MZ1 transformed with pREI (○) or pRE1-f310 (●) was solubilized with 6 M urea, renatured, and assayed for poly(A) polymerase activity as described. (B) The insoluble fraction from IPTG-induced cells of E. coli BL21 (DE3) transformed with pET28a (○) or pET28a-f310 (●) was solubilized, fractionated on Ni²⁺-Sepharose, and assayed for poly(A) polymerase activity as described.
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FIG. 4. SDS/PAGE analysis of proteins produced by E. coli strains transformed with an expression vectors carrying a f310 gene–hexahistidine fusion. The transformed cells were grown and disrupted and the cell extracts were processed as described. SDS/PAGE was carried out in 12.5% polyacrylamide gels together with a mixture of proteins of known molecular weight, followed by staining with Coomassie blue. The overexpressed 36-kDa protein is indicated by arrows. (A) Extracts of IPTG-induced cells of E. coli BL21 (DE3) transformed with pET28a or pET28a-f310, before and after fractionation into a soluble and inclusion body (pellet) fractions by centrifugation at 20,000 × g for 15 min. (B) The inclusion body (pellet) fractions from A were solubilized and fractionated on Ni²⁺-Sepharose, and equal proportions of the unbound (Ni wash) and the bound and eluted (Ni eluate) fractions were examined by SDS/PAGE.

DISCUSSION
In this report, we describe the disruption and identification of a candidate gene for a second poly(A) polymerase (PAP II) by an experimental strategy that was based on the assumption that the viability of E. coli depends on the presence of either PAP I or PAP II. The gene thus identified was the open reading frame f310, which is located at about 87 min on the E. coli chromosome adjacent to polA but oriented in the opposite direction (9). The following lines of evidence support the idea that f310 is indeed the gene for PAP II: (i) the deduced peptide encoded by f310 has a molecular weight of 36,300, which is similar to the molecular weight of 35,000 estimated by gel-filtration of PAP II (8); (ii) the deduced f310 product is a relatively hydrophobic peptide with a pI of 9.4, consistent with the properties of partially purified PAP II, which binds strongly to hydrophobic matrices such as phenyl agarose, eluting only with difficulty and in low yield (8), and which adsorbs to sulfopropyl Sepharose but not to DEAE Sepharose, at neutral pH (M. Kalapos and N.S., unpublished results); (iii) overexpression of f310 in appropriate expression vectors led to the formation of inclusion bodies whose solubilization and renaturation yielded substantial poly(A) polymerase activity, (iv) SDS/PAGE of the proteins from cells transformed with pRE1-f310 followed by enzyme blotting revealed a single overexpressed poly(A) polymerase activity with a molecular weight or 35,000; and (v) expression of a f310 fusion construct with hexahistidine at the N terminus of the coding region allowed purification of a poly(A) polymerase fraction whose major component (85%) was a 36-kDa protein. The specific activity of the purified poly(A) polymerase fusion protein was 40 units per mg of protein, about 25% of that of the 1000-fold purified preparation of PAP II obtained earlier (8). The lower specific activity of the fusion protein could have been due to some irreversible inactivation during denaturation of the inclusion bodies and metal ion chromatography, which had to be done in the absence of thiols, considering that PAP II is a relatively unstable enzyme (8), and perhaps also to the presence of the N-terminal hexahistidine moiety, whose effect on enzyme activity cannot be predicted.

The deduced 310-residue polypeptide product of f310 has a relatively high percentage of hydrophobic amino acids, many of which are concentrated in the 50 N-terminal residues as shown by the hydrophilicity plot (Fig. 5). The high degree of hydrophobicity and the clustering of the hydrophobic residues may be responsible for the tendency of the overexpressed product of the f310 gene to form inclusion bodies. Another notable feature is the relatively large excess of basic over acidic amino acids; as a result, the protein has a predicted pI of 9.4. Comparison of the deduced amino acid sequence of the f310 gene product with the protein sequence data banks revealed no significant homology with any known protein. On the other hand, search for possible motifs with functional significance uncovered two interesting features. One of these are two sets of paired cysteine and histidine residues: residues 67–80, CWCEGLAVLHLNPH; and residues 101–119, CNHRSWADIVVLCLFRKH. These clusters of cysteine and histidine residues are reminiscent of the Zn²⁺-binding domains

FIG. 5. Structure predictions for PAP II, the deduced f310 gene product. Hydrophilicity was estimated by the method of Kyte and Doolittle (21) and the secondary structure predictions were based on the method of Garnier et al. (22), utilizing the LASERGENE software package of DNastar (Madison, WI). Also shown are the disposition of basic and acidic amino acid residues and the location of the putative zinc fingers. Amino acids are numbered starting at the methionine at nucleotide 157 (Fig. 1).
often seen in nucleic acid-binding proteins and in hormone receptor proteins and referred to as zinc-fingers (23). The fact that the amino acids surrounding the cysteine/histidine pairs are not strictly required for the interaction of the zinc fingers of DNA binding proteins (24), especially with respect to spacing of the first Zn\(^{2+}\) binding pair, may be related to different structural requirements for the interaction of poly(A) polymerase with a single strand of RNA. In this connection, it is interesting that the zinc-binding domain of the glutamy RNA synthetases of \textit{E. coli} and several other bacterial species, which has recently been shown to be involved in tRNA binding (25), consists of the sequence C\textsc{CJC}\textsc{X2}\textsc{CRRH} (26). Another example of a zinc finger with only a single amino acid residue between the upstream cysteine pair is zinc finger 3 of yeast transcription factor SWI5 (27). The other feature of the deduced \textit{f310} translation product that resembles a known structural motif is the hydrophobic N-terminal segment. The 12 N-terminal residue, which include 2 basic amino acids, are followed by 9 nonpolar residues (67% alanine plus leucine) with \(\alpha\)-helical tendency (Fig. 5), similar to the "N" and "H" domains of the N-terminal signal peptides of the bacterial secretory protein precursors (28). However, whereas the signal peptide "H" domain is generally followed by a "C" domain, which contains the recognition sequence for signal peptidases, and then by a stretch of hydrophilic and acidic amino acids, the \textit{f310} product continues with almost 40 additional hydrophobic and basic amino acid residues. It is therefore unlikely that this extensive hydrophobic and cationic N-terminal domain is a signal sequence for protein secretion and its function in the context of poly(A) polymerase remains to be elucidated.

A common feature of the genes encoding PAP I and PAP II are relatively weak transcription or translation initiation elements, which may serve to keep the levels of expression low to avoid possible toxic effects of the kind observed when PAP I is overexpressed (2). Examination of the \textit{f310} sequence suggests the most likely translation start site for PAP II to be the AUG codon at position 157 (Fig. 2), which, although followed by the favorable sequence GCUA (29), is not preceded by a discernible ribosome binding site. (Possible alternate translation initiation codons, such as UUG at position 134 or AUG at position 184 are also not associated with recognizable ribosome binding sites.) In comparison, PAP I synthesis starts with the unfavorable UUG initiation codon and is preceded by a very weak ribosome binding site (2). As far as transcription initiation of \textit{f310} is concerned, no sequence that fits the canonical \(-10\) and \(-35\) elements for the major \textit{E. coli} RNA polymerase can be discerned upstream of the putative coding region, even though transcription must start between \textit{f310} and the beginning of the divergently transcribed \textit{polD} gene. It is interesting that the \textit{E. coli} \textit{duaG} gene, which encodes RNA primase, a protein produced only in very small amounts, also lacks discernible promoter and ribosome binding sites (30).

There is no sequence homology between the two \textit{E. coli} poly(A) polymerases, PAP I and PAP II, nor between the \textit{E. coli} enzymes and those from eukaryotes and viruses, suggesting independent origins and convergent evolution in terms of function. Indeed, PAP I of \textit{E. coli}, besides being capable of polyadenylating mRNA (31, 32), also functions in the replication control of plasmids of the ColE1 type by transferring adenylate residues to the 3′ ends of RNA I, the antisense repressor of plasmid DNA replication, thereby accelerating its degradation by polynucleotide phosphorylase (4, 5). The fact that the copy number of ColE1 plasmids is greatly reduced in \textit{penB} mutants (1) suggests that this regulatory function depends uniquely on PAP I and that PAP II cannot take its place. The question whether PAP II has unique functions which cannot be fulfilled by PAP I cannot be answered until strains are available in which the gene for PAP II has been specifically disrupted; however, the hydrophobic N-terminal domain of PAP II suggest the possibility of a membrane-associated function. On the other hand, there is no question that there is also considerable redundancy in the mRNA polyadenylating function of the two \textit{E. coli} poly(A) polymerases, since disruption of the \textit{penB} gene encoding PAP I has only modest effects on the extent of mRNA polyadenylation (8). Indeed, redundancy with respect to the role of signal sequence on RNA I seems almost to be a rule in \textit{E. coli}. For example, 3′-exonucleolytic degradation of mRNA can be effected both by polynucleotide phosphorylase and RNase II, so that mutual inactivation of either enzyme can be tolerated, but not the loss of both (10). Similarly, there is a high degree of functional overlap among the 3′ exoribonucleases involved in tRNA maturation, which include RNase D, RNase BN, RNase T, and RNase PH (33). The recent discovery of \textit{rnbH}, a gene encoding a second ribonuclease H (34) is another example of such functional duplication. Most likely, such redundancy serves in part as a fail-safe mechanism to guard against the loss of a vital function. Ironically, this functional and genetic redundancy has greatly hampered the genetic analysis of mRNA metabolism in \textit{E. coli} and is primarily responsible for our ignorance of this vital metabolic area. The identification of the gene for the second \textit{E. coli} poly(A) polymerase opens the way for the detailed investigation of the metabolic role of mRNA polyadenylation by studying the consequences of disruption of either or both of the poly(A) polymerase genes.

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