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The *Bacillus subtilis* Nucleotidyltransferase Is a tRNA CCA-Adding Enzyme

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There has been increased interest in bacterial polyadenylation with the recent demonstration that 3′ poly(A) tails are involved in RNA degradation. Poly(A) polymerase I (PAP I) of *Escherichia coli* is a member of the nucleotidyltransferase (Ntr) family that includes the functionally related tRNA CCA-adding enzymes. Thirty members of the Ntr family were detected in a search of the current database of eubacterial genomic sequences. Gram-negative organisms from the β and γ subdivisions of the purple bacteria have two genes encoding putative Ntr proteins, and it was possible to predict their activities as either PAP or CCA adding by sequence comparisons with the *E. coli* homologues. Prediction of the functions of proteins encoded by the genes from more distantly related bacteria was not reliable. The *Bacillus subtilis papS* gene encodes a protein that was predicted to have PAP activity. We have overexpressed and characterized this protein, demonstrating that it is a tRNA nucleotidyltransferase. We suggest that the *papS* gene should be renamed *cca*, following the notation for its *E. coli* counterpart. The available evidence indicates that *cca* is the only gene encoding an Ntr protein, despite previous suggestions that *B. subtilis* has a PAP similar to *E. coli* PAP I. Thus, the activity involved in RNA 3′ polyadenylation in the gram-positive bacteria apparently resides in an enzyme distinct from its counterpart in gram-negative bacteria.

mRNA polyadenylation now appears to be a common property of all living cells, but until recently it had been extensively studied only in eukaryotes (reviewed in references 9, 41, and 49). In the eubacteria, polyadenylated mRNAs have been detected in both gram-positive and gram-negative organisms (reviewed in references 43 and 44). Although an *Escherichia coli* poly(A) polymerase (PAP) activity was first described over 30 years ago (4), a convincing demonstration that polyadenylation is a general feature of prokaryotic messages was difficult because the poly(A) tails are short, mRNA turnover is very rapid, and only a fraction of the mRNAs are polyadenylated. The recent purification of *E. coli* PAP I and the identification of its gene (7) has led to increased interest in bacterial polyadenylation. PAP I is a 50-kDa protein encoded by the *pcnB* gene, which has been implicated in the control of plasmid copy number (30, 31, 33). The polyadenylation of RNA I, a plasmid-encoded antisense RNA that regulates ColE1 plasmid replication, mediates its degradation via a 3′ exonucleolytic pathway (23, 50, 51). Poly(A)-mediated exonucleolytic degradation has also been described for the antisense RNAs that regulate R1 plasmid replication and partition (36, 45), and it is believed to be involved in the degradation of mRNA (8, 21, 22, 37).

A second putative PAP of 35 kDa (PAP II), encoded by the open reading frame *f310*, has been identified in *E. coli* (6, 27). Strains with a disruption of the gene encoding either PAP I or PAP II are viable, suggesting a possible functional redundancy. However, there is no obvious sequence homology between the two *E. coli* polymerases, and PAP II is not related to any other known protein sequence. We previously detected proteins related to PAP I in a variety of bacteria, including *Bacillus subtilis*, *Desulfovibrio gigas*, and *Proteus mirabilis*, using an antibody raised against the purified *E. coli* PAP I (39). PAP I is a member of the X polymerase family, which includes the eukaryotic PAPs as well as all of the known nucleotidyltransferases (34, 52). The PAP I and tRNA nucleotidyltransferase of *E. coli* have extensive homology in their N-terminal halves (35). Their similarity to the eukaryotic PAPs is limited to a small number of conserved residues that are critical for activity (34, 39a). The tRNA 3′ CCA-adding activity is specific to the tRNA nucleotidyltransferases (reviewed in reference 14). In eukaryotes, where the CCA is rarely encoded by the tRNA gene, the tRNA nucleotidyltransferase is essential (1). In bacteria, the 3′ CCA is generally encoded by the tRNA gene. Nevertheless, the *E. coli* CCA-adding enzyme has an important role in the repair of tRNA 3′ ends, and its inactivation significantly slows growth (11, 16, 53).

Although the *E. coli* tRNA nucleotidyltransferase can add CCA or repair ends (CA or A addition), we know of no evidence that it can add longer 3′-terminal extensions (Fig. 1A). In contrast, PAP I in vitro can add poly(A) tails several hundred nucleotides long to tRNA or other RNA substrates (Fig. 1B) (39). Although the principal role of PAP I is believed to be 3′ polyadenylation, PAP I and polynucleotide phosphorylase can repair partially damaged tRNA CCA ends in *E. coli* mutants that are deficient for CCA-adding activity (40). In this paper, we show that, like *E. coli*, other organisms from the β and γ subdivisions of the purple bacteria have two genes encoding proteins that can be classified as either a tRNA nucleotidyltransferase or PAP by protein sequence comparisons. However, prediction of the activity of Ntr proteins encoded by genes from more distantly related bacteria is not reliable. The gram-positive bacterium *B. subtilis*, whose genome has now been completely sequenced, contains a single gene encoding an Ntr protein that we show is a tRNA nucleotidyltransferase. The apparent lack of an Ntr protein with PAP activity is surprising because previous evidence suggested that *B. subtilis* has an activity similar to that of *E. coli* PAP I.

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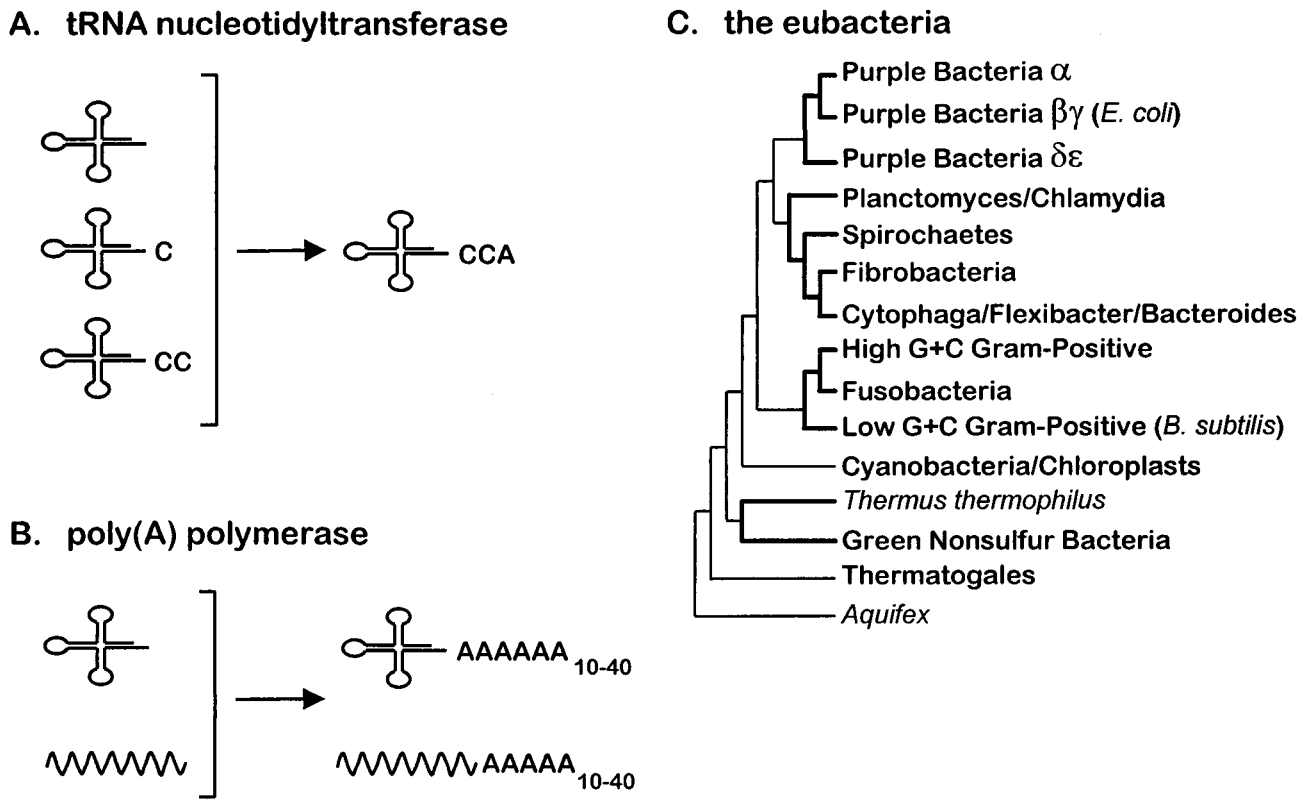

FIG. 1. (A) Repair of tRNA CCA ends by nucleotidyltransferase; (B) 3′ poly(A) addition to tRNA or other RNA substrates by PAP; (C) phylogenetic tree showing the relationship of the major families of eubacteria (38). *Thermus thermophilus* and *Aquifex* are single organisms.

MATERIALS AND METHODS

The construction of pET11a-derived vectors for protein expression and the preparation of protein extracts was done as described previously (39). Briefly, the DNAs encoding the *E. coli cca* and *B. subtilis papS* open reading frames were PCR amplified with the oligonucleotide pairs 5′ATATGAAGATTTATCTGG TCGGTGGTGC3′ and 5′TCATTCAGGCTTTGGGCAAGCTTGTTCC3′ (*E. coli*) or 5′ATATGGAAAAAGTTTTTATCAAAGCACTTCC3′ and 5′TTAAT GTTAGACCGCATGTCTTCAGCC3′ (*B. subtilis*) with genomic DNA from *E. coli* D10 (20) or *B. subtilis* QB936 (13). In the construction expressing the *E. coli* CCA-adding enzyme, we changed the GTG initiation codon to ATG. Protein expression was done in the BL21(DE3) strain with 1 mM IPTG (isopropyl-β-D-thiogalactopyranoside) induction for 2 h. Cultures, concentrated fivefold, were suspended in a lysis buffer and sonicated. The extracts were treated with 10 μg of DNase I/ml and then adjusted to 0.8 M NH_4Cl. The cell debris and the ribosomes were removed by centrifugation. The purities of the overexpressed proteins were estimated with a Coomassie blue-stained sodium dodecyl sulfate (SDS)-polyacrylamide gel as follows: *E. coli* PAP I, 10%; *E. coli* tRNA CCA-adding enzyme, 2%; and *B. subtilis* PapS, 30%. The low level of the CCA-adding enzyme was due to its inefficient expression. In the assays described below, 250 (PAP I), 1,250 (CCA-adding enzyme), or 80 ng (PapS) of total protein was used to give equivalent amounts of each overexpressed protein (25 ng). For ease of comparison, the values for normalized specific activities (see Table 2) are based on the estimated amounts of the overexpressed proteins.

The following conditions were used to measure the specific activities (see Table 2). The tRNA nucleotidyltransferase assay was done as described previously (15). The protein extracts were diluted in 10 mM glycine (pH 9.4)–1 mg of *Saccharomyces cerevisiae* tRNA/ml. Reaction mixtures (200 μl) containing 50 mM glycine (pH 9.4), 5 mM $MgCl_2$, 500 μM ATP, 10 μCi of [α-^{32}P]ATP (or [α-^{32}P]CTP), 150 μg of yeast tRNA or poly(A), and 25 ng of overexpressed protein were incubated for 10 min at 37°C. In the gel analysis of the tRNA addition products (see Fig. 4), the reaction mixtures were the same except that the volume was reduced to 20 μl. The PAP assay was done as described previously (39). The protein extracts were diluted in a solution containing 10 mM Tris-HCl (pH 7.5), 500 mM NaCl, 5% glycerol, 0.5% Triton X-100, 1 mM EDTA, and 1 mM dithiothreitol. Reaction mixtures (200 μl) containing 10 mM Tris-HCl (pH 7.5), 100 mM NaCl, 1% glycerol, 0.1% Triton X-100, 4 mM $MgCl_2$, 200 μM ATP, 20 μCi of [α-^{32}P]ATP, 4 μg of yeast tRNA, and 25 ng of the overexpressed protein were incubated for 15 min at 37°C.

PCR amplifications of genomic DNA with inosine-containing oligonucleotides were done with 100 ng of *B. subtilis* DNA with the oligonucleotide pair 5′GTIG GIGGI(C/G)I(A/G)TI(C/A)GIGA(T/C)3′ and 5′(G/A)TTIAIIGTIA(A/G)ITC IC(G/T)IT3′. Control amplifications were done with the pET11a-*pcnB* plasmid and the same oligonucleotides. The reactions (50 μl) with *Tub* DNA polymerase (Amersham) were performed under the following conditions: 95°C, 30 s; 46°C, 3 min; 66°C, 40 s; 50 cycles.

RESULTS

Nucleotidyltransferase proteins in the eubacteria. With many genomic sequencing projects finished or in progress, extensive comparisons among the eubacteria are now possible (Fig. 1C). Putative Ntr proteins have already been detected in bacteria such as *Haemophilus influenzae*, *Acidaminococcus fermentans*, *Aeromonas hydrophyla*, and *B. subtilis* (34, 52). We found 25 more proteins in a recent search (May 1998), and their number will surely increase as more genomes are completed. Table 1 summarizes the protein sequences detected in this search. All of these homologues share a conserved amino-terminal domain containing motifs that are characteristic of the Ntr protein family (34, 52). For the gram-negative organisms in the β and γ subdivisions of the purple bacteria, which include *E. coli*, two Ntr proteins were predicted. As indicated in Table 1, it was possible to classify the predicted proteins as either a PAP or tRNA nucleotidyltransferase (noted as CCA in Table 2) by comparison with the *E. coli* homologues. The alignment of these two groups of proteins is shown in Fig. 2. The remainder of the proteins identified in Table 1 cannot be

TABLE 1. Putative Ntr proteins in the eubacteria[a]

Organism	Group[b]	Genome sequence	Protein size (amino acids)[c]	Protein activity[d]	Reference
E. coli	PBγ	Complete	473	PAP	5
			412	CCA	
H. influenzae	PBγ	Complete	488	PAP	17
			416	CCA	
Pseudomonas aeruginosa	PBγ	Partial	408*	PAP	University of Washington
			185*	CCA	
Actinobacillus actinomycetemcomitans	PBγ	Partial	259*	PAP	University of Oklahoma
			207*	CCA	
Neisseria gonorrhoeae	PBβ	Partial	470	PAP	University of Oklahoma
			366*	CCA	
Neisseria meningitidis	PBβ	Partial	375*	PAP	University of Oklahoma
			399	CCA	
A. hydrophyla	GN	Partial	203*	CCA	26
Francisella tularencis	GN	Partial	84*	CCA	NCBI[a]
Chlamydia trachomatis	GN	Partial	353*	?	University of California
			290*	?	
Helicobacter pylori	PBδε	Complete	411	?	48
A. fermentans	GN	Partial	343*	?	32
Campylobacter jejuni	GN	Partial	201*	?	Sanger Center
Rickettsia prowazekii	GN	Partial	149*	?	University of Uppsala
Borrelia burgdorferi	SP	Complete	410	?	18
B. subtilis	GP	Complete	403	?	29
M. tuberculosis	GP	Complete	482	?	Sanger Center
M. leprae	GP	Partial	411	?	19
Streptococcus pyogenes	GP	Partial	199*	?	University of Oklahoma
Staphylococcus aureus	GP	Partial	121*	?	University of Oklahoma
Thermus thermophilus		Partial	329*	?	3
Synechocystis	CB	Complete	420	?	28
			949	?	
Aquifex		Complete	512	?	12
			845	?	

[a] Putative Ntr proteins were identified by Blast (2) with the *E. coli* CCA-adding or PAP I protein sequence against databases described in published references or available at the following sites: University of Washington (www.genome.washington.edu), University of Oklahoma (www.genome.ou.edu), National Center for Biotechnology Information (NCBI) (www.ncbi.nlm.nih.gov), University of California (C. Fenner; ctgenome@socrates.berkeley.edu), Sanger Center (www.sanger.ac.uk), and University of Uppsala (www.unu.se).

[b] PB, purple bacteria; GN, gram negative; SP, spirochetes; GP, gram positive; and CB, cyanobacteria.

[c] Asterisks indicate that the protein was predicted from a partial coding sequence and the size is that of the incomplete protein.

[d] For bacteria other than *E. coli*, PAP or tRNA nucleotidyltransferase (CCA) activity was predicted from the sequence. For bacteria that are only distantly related to *E. coli*, the activity could not be predicted (?).

reliably classified by sequence alignment. This is illustrated in Fig. 2, where the *B. subtilis* Ntr protein sequence is aligned with the tRNA nucleotidyltransferase and PAP homologues. The colored boxes indicate the conserved amino acids. There are 28 residues conserved between the *B. subtilis* homologue and the tRNA nucleotidyltransferases, 37 residues in common with the PAPs, and 55 residues in common with both groups of proteins. The somewhat-better alignment with the PAPs led to the prediction that this protein is a PAP, and its gene was named *papS* (29).

Characterization of the *B. subtilis papS* gene. *B. subtilis*, a distant relative of *E. coli*, is a gram-positive organism that has been extensively studied. Because we were interested in characterizing PAP I-like enzymes, the *papS* gene was amplified by PCR and cloned, and the protein was expressed in *E. coli*. Using the genomic sequence, we chose the longest open reading frame, which begins with a methionine and encodes a protein of 403 amino acids. In the same manner, we cloned and expressed the *E. coli* CCA-adding enzyme, which, together with *E. coli* PAP I, served as a control for the following experiments. The overexpressed *B. subtilis* PapS protein migrated on SDS-polyacrylamide gels at the expected molecular mass of 45 kDa (Fig. 3A, lane 5). It was somewhat smaller than the *E. coli* PAP I, which migrates as a 50-kDa protein (Fig. 3A, lane 3). Figure 3B, a Western blot with an antibody against *E. coli* PAP I, shows the expected reaction with overexpressed PAP I (lane 3). In Fig. 3B, lanes 1 and 4, the protein that is somewhat smaller than the overexpressed PAP I is the endogenous *E. coli* PAP I that is processed by removing 17 amino acids from its amino terminus (7, 39). The faintly detected protein in Fig. 3B, lane 2, which is slightly larger than the overexpressed PAP I, is the *B. subtilis* protein that cross-reacts with the PAP I antibody (39). Note that PapS (Fig. 3B, lane 5), like the *E. coli* CCAase (Fig. 3B, lane 4), does not cross-react with the PAP I antibody, and it is smaller than the *B. subtilis* protein (Fig. 3B, lane 2). Thus, the *papS* gene product characterized here does not appear to be related to the *B. subtilis* protein that cross-reacts with the antibody against *E. coli* PAP I.

The activity of overexpressed PapS was measured under different assay conditions to discriminate between tRNA CCA addition and PAP activity. *E. coli* PAP I has detectable activity under both neutral and basic conditions (pHs 9 to 10), whereas the tRNA CCA-adding enzyme is only active under basic conditions (our data and reference 15). In these assays, either crude yeast tRNA, which contains molecules missing part or all of the CCA terminus, or poly(A) was used as the acceptor. The results shown in Table 2 indicate that the *B. subtilis papS* gene product has the same properties as the *E. coli* tRNA nucleoti-

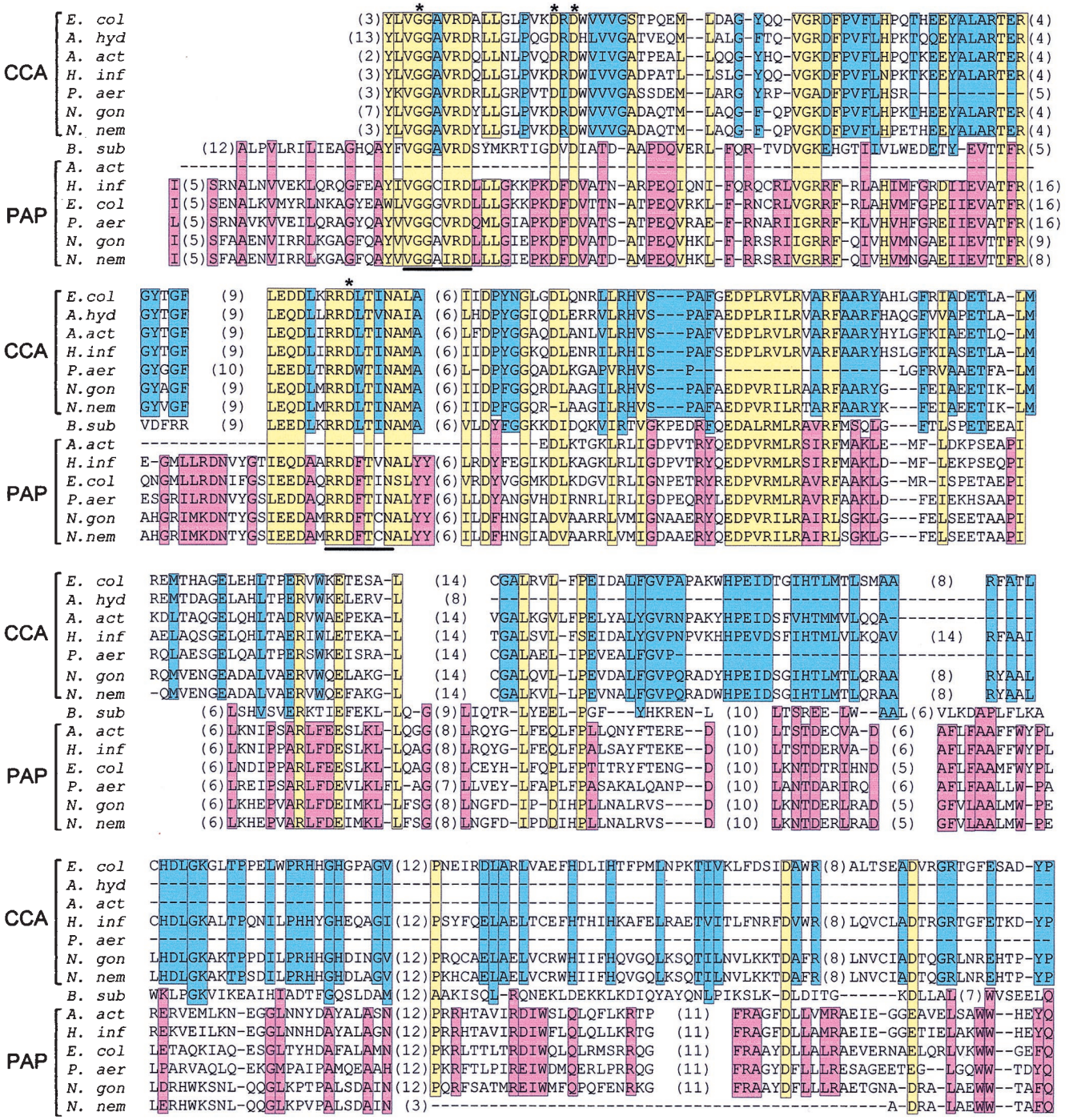

FIG. 2. Sequence alignment of the tRNA nucleotidyltransferase (CCA) and PAP I (PAP) homologues from members of the β and γ subdivisions of the purple bacteria (*E. col*, *E. coli*; *A. hyd*, *A. hydrophila*; *A. act*, *Actinobacillus actinomycetemcomitans*; *H. inf*, *H. influenzae*; *P. aer*, *Pseudomonas aeruginosa*; *N. gon*, *Neisseria gonorrhoeae*; *N. nem*, *Neisseria meningitidis*) with the related protein from *B. subtilis* (*B. sub*). The initial alignment was made with CLUSTAL (24, 25), and small realignments were made manually. The colored boxes indicate the positions of conserved amino acids of the CCA-adding family (blue), the PAP I family (pink), and both families (yellow). The asterisks show the conserved G-D-D-D residues that are the signature of the X polymerase family. The protein motifs used to design the degenerate inosine-containing oligonucleotides are underlined. The numbers in parentheses represent amino acids not shown in the alignment, and dashes represent gaps in the alignment.

dyltransferase, with a comparable specific activity (published values for the *E. coli* enzyme range from 1,000 to 8,000 U/mg, depending on the preparation [14]). Like the *E. coli* CCA-adding enzyme, PapS adds AMP or CMP specifically to the tRNA acceptor, and it has no detectable activity under the polyadenylation assay conditions. As expected, PAP I, which is active under both assay conditions, only adds AMP and can use tRNA or poly(A) as an acceptor.

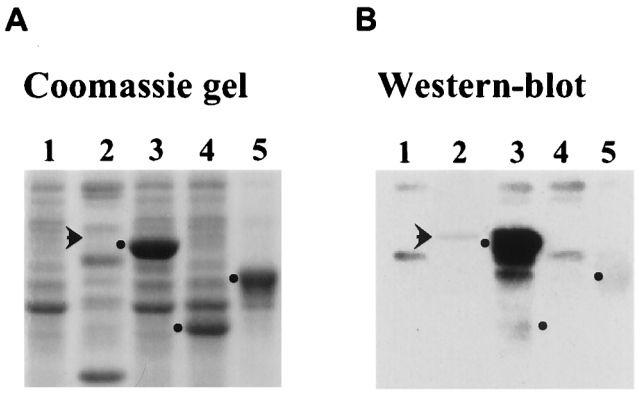

FIG. 3. Expression and Western blot analysis of the *B. subtilis* PapS protein. Proteins were separated by SDS-polyacrylamide gel electrophoresis, and the gel was stained with Coomassie blue (A) or analyzed by Western blotting with the *E. coli* PAP I antibody (B). Lanes 1 and 2, *E. coli* and *B. subtilis* total proteins. Lanes 3 to 5, extracts of PAP I, *E. coli* tRNA nucleotidyltransferase, and *B. subtilis* PapS, respectively, were loaded to give similar amounts of each of the overexpressed proteins (see Materials and Methods). The dots in lanes 3 to 5 indicate the positions of the overexpressed proteins. The arrows in lanes 2 show the positions of a *B. subtilis* protein that cross-reacts with the PAP I antibody.

In Fig. 4, the products of the reaction under the CCA assay conditions with yeast tRNA and either [α-^{32}P]ATP (Fig. 4A) or [α-^{32}P]CTP (Fig. 4B) were characterized by electrophoresis on a denaturing polyacrylamide gel. *E. coli* PAP I (Fig. 4, lanes 1) elongated the tRNA, adding at least 400 AMP residues (Fig. 4A), but it is unable to incorporate CMP (Fig. 4B). Figure 4, lanes 2, contains a control mock preparation from cells without protein overexpression. Figure 4, lanes 3 and 4, shows the CCA-adding activity of the *E. coli* and *B. subtilis* enzymes, respectively. The molecules in the yeast tRNA population are extended to CCA (Fig. 4A) or to C and CC (Fig. 4B). The background in the high-molecular-weight regions of all the lanes in Fig. 4B is specific to the [α-^{32}P]CTP label. The radiolabeled tRNA population in Fig. 4, lanes 3 and 4, is heterogeneous due to the size distribution of the yeast tRNAs (72 to 95 nucleotides [46]). Comparable profiles were observed when crude wheat germ tRNA was employed to characterize the CCA-adding enzyme from *Sulfolobus shibatae* (52). In all the data shown in Table 2 and Fig. 4, the activity of the *B. subtilis* PapS protein is indistinguishable from that of the *E. coli* tRNA nucleotidyltransferase. Thus, we conclude that PapS is a tRNA CCA-adding enzyme.

TABLE 2. Activity of the *B. subtilis* PapS protein

Assay conditions[a]	Substrates	Normalized specific activity (U/mg)[b]		
		PAP I (*E. coli*)	CCA (*E. coli*)	PapS (*B. subtilis*)
CCA addition	tRNA, ATP	1,510	910	880
	tRNA, CTP	0	1,530	1,490
	poly(A), ATP	520	0	0
Polyadenylation	tRNA, ATP	2,820	0	0

[a] Incorporation of radioactive AMP or CMP was assayed by trichloroacetic acid precipitation under CCA addition or polyadenylation assay conditions (see Materials and Methods).

[b] Values, based on the assay of protein extracts, have been normalized to the amount of overexpressed protein (see Materials and Methods). For each determination, a background, which ranged from 10- to 100-fold lower than the signal, was subtracted. The background reactions used an equivalent amount of total protein prepared from the BL21(DE3) strain with the pET11a expression vector. The units of activity are micromoles of AMP (or CMP) incorporated per hour in the CCA addition assay and nanomoles of AMP incorporated per 10-minute interval in the polyadenylation assay.

Is there another gene encoding an Ntr protein in *B. subtilis*? The *B. subtilis* genome apparently contains only one gene encoding an Ntr protein, which we have now identified as a CCA-adding enzyme. However, a second gene may have been missed because of an error in the genomic sequence. About 4,200 genes have been identified in *B. subtilis*, and it has been estimated that another 100 to 200 genes will be identified as the genomic sequence is corrected (29). We tried two approaches to identify a second gene. First, we screened a λGT11 expression library, derived from *B. subtilis* (47), using our antibody against *E. coli* PAP I. Over 80,000 plaques were examined, but we failed to detect a protein clearly related to *E. coli* PAP I. In a second approach, we used a pair of inosine-containing oligonucleotides designed to hybridize to the regions encoding two highly conserved protein motifs in the eubacterial Ntr proteins (Fig. 2). Pilot experiments demonstrated that these primers could amplify DNA fragments of the correct size with templates containing the cloned *E. coli pcnB* or *cca* genes. In Fig. 5, a 260-bp DNA fragment amplified from the *B. subtilis* genomic DNA (lane 1), which was slightly smaller than the 290-bp DNA fragment from the cloned *E. coli pcnB* gene (lane 2), was detected. The genomic PCR product had the size predicted for the known *B. subtilis papS* gene, and restriction digestion confirmed this identification (data not shown). Under the PCR conditions employed here, several products of 450 bp or larger were detected due to the hybridization of the degenerate oligonucleotides to nonspecific sites. These products are too large to correspond to an Ntr coding sequence. Thus, we could not detect another gene by this method.

DISCUSSION

Our antiserum against *E. coli* PAP I was previously shown to detect proteins in several other gram-negative bacteria: *Yersinia pseudotuberculosis*, *Erwinia carotovora*, *P. mirabilis*, and *D. gigas* (39). We also detected a related protein in *B. subtilis*, a member of the low-G+C gram-positive family of bacteria (Fig. 1C). By Western blotting, the *B. subtilis* protein, with a mass of 55 kDa, reacted weakly with the antibody against *E. coli* PAP I, requiring a 10-fold-longer exposure for detection by chemiluminescence (39). The 45-kDa protein characterized here, which is the *B. subtilis* tRNA nucleotidyltransferase, does not react with the antibody against *E. coli* PAP I. The apparent lack of a PAP I homologue in *B. subtilis* suggests that the weak signal detected with the antibody against *E. coli* PAP I is due to a cross-reaction with an unrelated protein.

The genome of *Mycobacterium tuberculosis*, another gram-positive bacterium, has recently been completely sequenced, and like that of *B. subtilis*, it contains a single gene encoding a putative Ntr protein (Table 1). We have expressed and characterized the closely related protein from *Mycobacterium leprae* and found that it is also a tRNA nucleotidyltransferase (unpublished results). The finding that *B. subtilis* and *M. tuberculosis* each contain a single gene encoding a CCA-adding enzyme suggests that PAP I is either an ancient enzyme which has been lost in certain bacteria or that it a new enzyme in the purple bacteria arising from a recent duplication of the tRNA nucleotidyltransferase gene. It should be feasible to distinguish between these two possibilities when there is more information about the distribution of the CCA-adding and PAP I-like enzymes among the eubacteria.

Most of our understanding of RNA processing and degra-

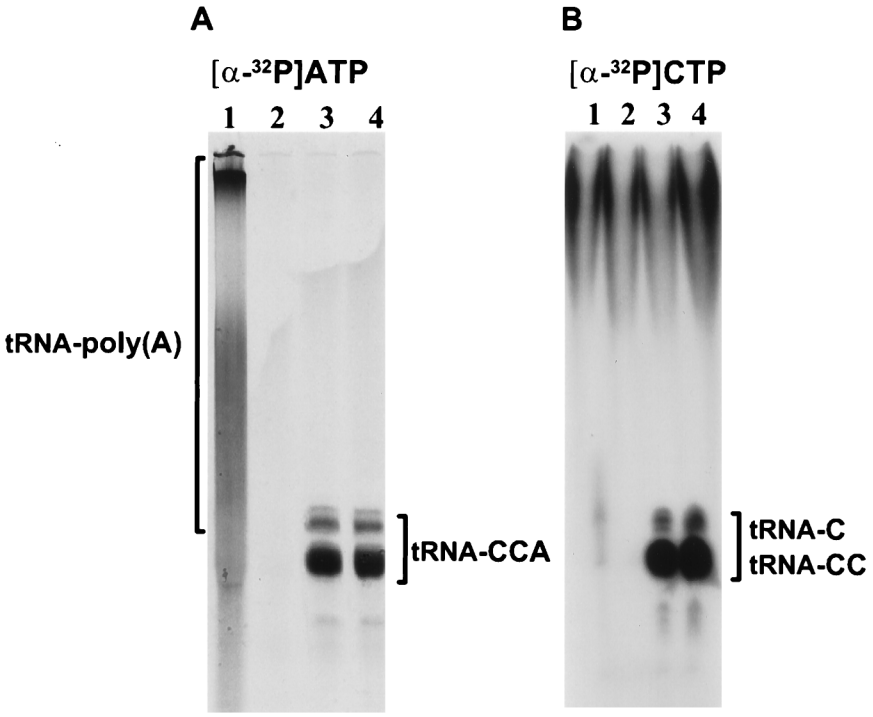

FIG. 4. Polyacrylamide gel analysis of products under CCA assay conditions with yeast tRNA as the acceptor and [α-^{32}P]ATP (A) or [α-^{32}P]CTP (B) as the substrate. Lanes: 1, *E. coli* PAP I; 2, protein extract from BL21(DE3) with the pET11a expression vector; 3, *E. coli* tRNA nucleotidyltransferase; 4, *B. subtilis* PapS.

dation in the eubacteria comes from the study of *E. coli*. Of the proteins predicted by genomic sequencing, *B. subtilis* lacks RNase E, an endonuclease known to be important in *E. coli* rRNA processing and mRNA decay. Nevertheless, an RNase E-like activity has been suggested based on the study of the endonucleolytic processing of a tRNA synthetase message (10). When the tRNA synthetase gene from *B. subtilis* was transferred and expressed in *E. coli*, the message was processed at the same sites as in *B. subtilis* in an RNase E-dependent reaction. It was also correctly processed in vitro with purified RNase E. A related observation is that two PAP activities, suggested to be similar to *E. coli* PAP I and PAP II, have been described in *B. subtilis* (42). Thus, the failure to detect clearly discernible RNase E and PAP homologues in *B. subtilis* is unexpected, raising the possibility that these activities reside in proteins distinct from their counterparts in gram-negative bacteria.

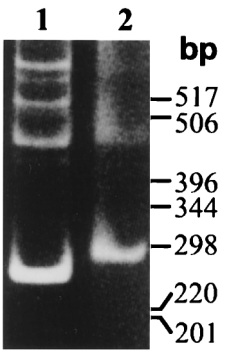

FIG. 5. PCR amplification of the *papS* gene from *B. subtilis* genomic DNA with degenerate inosine-containing oligonucleotides designed to bind to DNA encoding two of the most highly conserved motifs in the eubacterial Ntr protein family (Fig. 2). Lane 1, *B. subtilis* genomic DNA; lane 2, cloned *E. coli pcnB* gene (in pET11a).

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