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Molecular Cloning of the *Escherichia coli* Gene for Diadenosine 5′,5‴-P^1,P^4-Tetraphosphate Pyrophosphohydrolase

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A clone overproducing diadenosine tetraphosphatase (diadenosine 5′, 5‴-P^1, P^4-tetraphosphate pyrophosphohydrolase) activity was isolated from an *Escherichia coli* cosmid library. Localization of the DNA region responsible for stimulation of this activity was achieved by deletion mapping and subcloning in various vectors. Maxicell experiments and immunological assays demonstrated that a 3.5-kilobase-pair DNA fragment carried the structural gene *apaH* encoding the *E. coli* diadenosine tetraphosphatase. The DNA coding strand was determined by cloning this fragment in both orientations in pUC plasmids. It was also shown that the overproduction of diadenosine tetraphosphatase decreased the dinucleoside tetraphosphate concentration in *E. coli* by a factor of 10.

The family of bis(5′-nucleosidyl)oligophosphates deserves increasing interest since the discovery of bis(5′-adenosyl)tetraphosphate (Ap_4A) in biological materials (26, 31) and the recent hypotheses that these nucleotides could be involved in the priming reaction of replication (9, and references therein, 30) or be synthesized as alarmones to signal the onset of cellular stress (3).

The unusual Ap_4N nucleotides (where N stands for any nucleoside) are produced in vitro by the aminoacyl-tRNA synthetases through the reversal of enzyme-bound aminoacyl-adenylate by NTP (32). In the case of a few synthetases, the rate of Ap_4N synthesis can be greatly enhanced on the addition of small amounts of zinc (2, 6, 8, 16, 25). There exists specific hydrolases for the catabolism of the Ap_4N nucleotides (1, 7, 10, 14, 17, 23, 24). In *Escherichia coli*, a single enzyme activity capable of hydrolyzing Ap_4N as well as Ap_3N has been characterized in vitro (10, 24). In this study advantage was taken of the characterization and the purification of this enzyme to clone its gene.

MATERIALS AND METHODS

E. coli strains and plasmids used in this study are listed in Table 1. General genetic and cloning techniques have been described previously (19, 21).

Preparation of the cosmid library. High-molecular-weight chromosomal DNA was prepared by the method of Harris-Warrick et al. (11).

To avoid the formation of pHC79 multimers, two arms of this vector were constructed. Cosmid pHC79 DNA was digested with either *Eco*RI or *Sal*I, phenol extracted, and ethanol precipitated, and the 5′ termini were dephosphorylated (Fig. 1). After phenol extraction and ethanol precipitation, each arm was cut with *Bam*HI. An equimolar mixture of these two arms (1 μg each) was ligated with 1 μg of chromosomal DNA from *E. coli* K37 partially restricted by *Sau*3A and dephosphorylated, in a final volume of 25 μl. After an overnight incubation at 14°C, 5-μl fractions were packaged by using a mixture of crude extracts prepared from strains BHB2688 and BHB2690 (12). Packaged particle suspensions were stored at 4°C. These suspensions were used to transduce strain IBPC111 (2×10^4 transductants per μg of chromosomal DNA). A total of 500 ampicillin-resistant transductants were purified further and stored at −80°C in LB medium containing 7% dimethyl sulfoxide.

Crude extract preparations and enzymatic assay. Crude extracts were prepared from 5-ml cultures which were obtained as follows. A single colony was inoculated into 0.25 ml of morpholine propanesulfonic acid (MOPS)-low glucose (0.04%) medium (22) supplemented with 0.2% Casamino Acids (Difco Laboratories, Detroit, Mich.) and 50 μg of ampicillin per ml and then was grown overnight at 37°C without shaking. This technique ensured a low growth rate and thus, after this overnight incubation, absorbance did not exceed an optical density of 0.3 at 650 nm. A total of 5 ml of MOPS-glucose (0.4%) supplemented with 0.2% Casamino Acids, 100 μg of ampicillin per ml, and 0.08 μCi of [^{14}C]Ile (135 mCi/mmol) per ml were then added to the 0.25-ml cultures (measurement of the radioactivity from labeled Ile incorporated in the proteins would be used as [illegible]apid determination of the protein concentrations in the [illegible]). Cultures were grown at 37°C under shaking, up to an [illegible] density at 650 nm of 0.4. Cells were harvested by cen[illegible]gation, washed with 1 ml of buffer A (20 mM Tr[illegible] hydrochloride [pH 7.6], 0.1 mM EDTA), pelleted, and stored at −20°C. Routinely, 100 cultures were carried out at the same time. This method limited the generation number and thus reduced recombinant cosmid segregation. In addition, it ensured a uniform physiological state of the different clones.

When needed, cell pellets (usually 25 in a set of assays) were allowed to thaw on ice and were suspended in 90 μl of buffer A supplemented with 0.1 mM dithioerythritol. Cell disruption was achieved by adding 2 μl of 1% sodium dodecyl sulfate (SDS) and 5 μl of chloroform. After 30 s of vigorous stirring, cell debris was removed by centrifugation.

Diadenosine tetraphosphatase activity was followed at 37°C by the radioisotopic assay described previously (24). Initial rates of hydrolysis were calculated from the amounts of adenosine synthesized in a reaction mixture (100 μl) containing 50 mM Tris-hydrochloride (pH 7.6), 50 μM [^{3}H]Ap_4A (20 Ci/mol; Amersham Corp., Arlington Heights, Ill.), 150 μM $CoCl_2$, an excess of calf intestine alkaline

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TABLE 1. Bacterial strains and plasmids

Strain and plasmids	Relevant genotype	Reference or source
Strains		
K37	*galK rpsL*	
IBPC111	F^- Δ(*lac-pro*) *gyrA rpoB metB argE*(Am) *supE ara recA1* λ^s λ^-	28
HB101	*pro leu hsdR hsdM recA*	5
BHB2688	N205 *recA* (λ *imm*^{434} cIts *b2 red-3 E*(Am)4 *S*(Am)7)	12
BHB2690	N205 *recA* (λ *imm*^{434} cIts *b2 red-3 D*(Am)15 *S*(Am)7)	12
CSR603	*recA1 uvrA6 phr-1 thr-1 leuB6 proA2 argE3 thi-1 ara-14 lacY1 galK2 xyl-5 mtl-1 rpsL31 tsx-33 supE44*	27
JM101	*supE thi* Δ(*lac-pro*) F′ (*traD36 lacI*^{q} *proAB lacZ*ΔM15)	20
Plasmids		
pHC79	Ap^rTc^r derivative of pBR322 carrying the *cos* sequence of λ	13
pOS47	Ap^r *apaH* derivative of pHC79	This study
pOS47S12D	Ap^r *apaH* derivative of pHC79	This study
pBR322	Ap^r Tet^r	4
pAP47	Ap^r *apaH* derivative of pBR322	This study
pUC12	Ap^r carries the beginning of *lacZ*	29
pUC13	Ap^r carries the beginning of *lacZ*	29
pUC1247	Ap^r *apaH* derivative of pUC12	This study
pUC1347	Ap^r *apaH* derivative of pUC13	This study
pUC1247BPD	Ap^r *apaH* derivative of pUC1247	This study
pUC1247B	Ap^r derivative of pUC1247	This study

phosphatase (30 U/ml; Boehringer GmbH, Mannheim, Federal Republic of Germany), and 20 μl of the crude extract. When necessary, the extract was diluted in buffer A supplemented with 200 μg of bovine serum albumin per ml and 0.1 mM DTE prior to the assay. In addition, a 40-μl fraction of each extract was precipitated with trichloracetic acid and filtered through GF/C filters (Whatman, Inc., Clifton, N.J.). The radioactivity from [^{14}C]Ile retained on the filters was counted in an Intertechnique SL32 spectrometer. This measurement reflected the protein concentration in the extracts; therefore, specific activities were expressed as the ratio of diadenosine tetraphosphatase activity (1 U corresponding to 1 nmol of Ap_4A hydrolyzed per min at 37°C) over the trichloracetic acid-precipitable radioactivity in 40 μl of the extract.

Identification of plasmid-encoded proteins in maxicells. CSR603 cells transformed by the appropriate plasmid were UV irradiated (≃50 J/m^2) under agitation and incubated overnight after the addition of 150 μg of cycloserine per ml to kill survivors. Cells were washed and suspended in minimal medium and labeled for 1 h at 37°C with 10 μCi of L-[^{35}S]methionine (1,200 Ci/mmol; CEA Saclay). Bacteria were collected by centrifugation, and lysis was obtained by the freeze-thaw procedure. Samples were then either submitted directly to SDS-polyacrylamide gel electrophoresis (PAGE) or incubated with antibodies, precipitated by protein A-Sepharose (Pharmacia Uppsala, Sweden), and washed before heat denaturation and SDS-PAGE.

Preparation of specific antibodies. Homogenous diadenosine tetraphosphatase (24) was used to immunize white New Zealand rabbits for antibody production. Each rabbit received a 2-ml mixture containing 150 μg of diadenosine tetraphosphatase and 1 ml of complete Freund adjuvant. This mixture was inoculated by intraperitoneal and subcutaneous injections in the neck and thigh. Two additional inoculations were performed 3 weeks and 5 weeks after the primary injection. The highest titer was obtained 1 week after the third injection. Rabbits were bled through heart puncture. Immunoglobulins were then purified by filtration through a Sephadex G200 column after ammonium sulfate (45% saturation) precipitation and dialysis against 20 mM Tris-hydrochloride (pH 7.8) supplemented with 10 mM 2-mercaptoethanol. Immunoglobulins were stored at 4°C as a

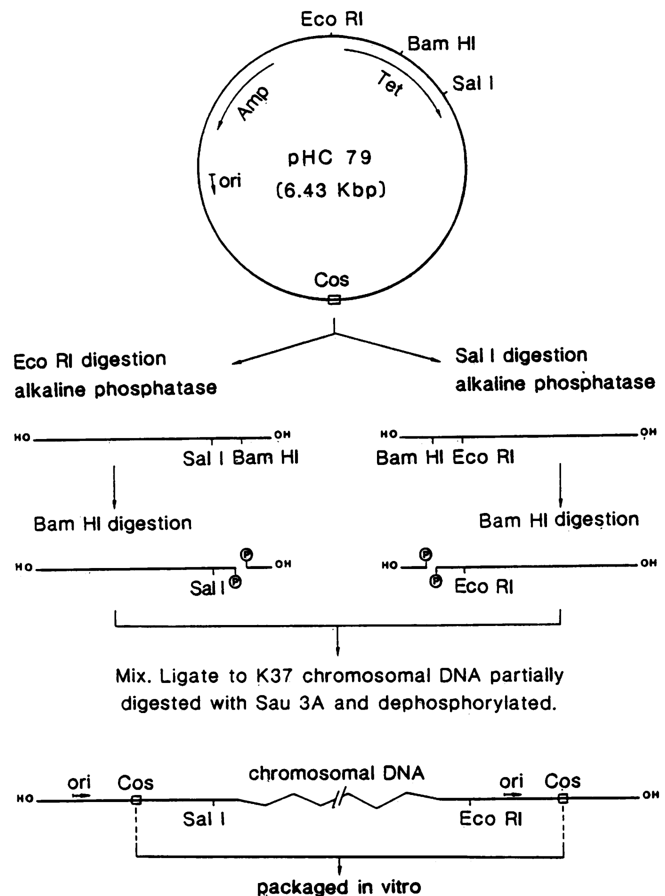


FIG. 1. Construction of an *E. coli* K-12 cosmid library. The *cos* sequence, the relevant restriction sites, and the origin of replication of pHC79 are indicated. pHC79 DNA was digested by either *Eco*RI or *Sal*I. After dephosphorylation, these two arms were cut by *Bam*HI. A total of 1 μg of each arm was mixed with 1 μg of K37 chromosomal DNA partially restricted with *Sau*3A and dephosphorylated. A total of 4 U of T4 DNA ligase was added to this 25-μl mixture. After an overnight incubation at 14°C, recombinant cosmids were packaged in vitro. A total of 500 ampicillin transductants of strain IBPC111 were purified three times and stored at −80°C in LB medium containing 7% (vol/vol) dimethyl sulfoxide.

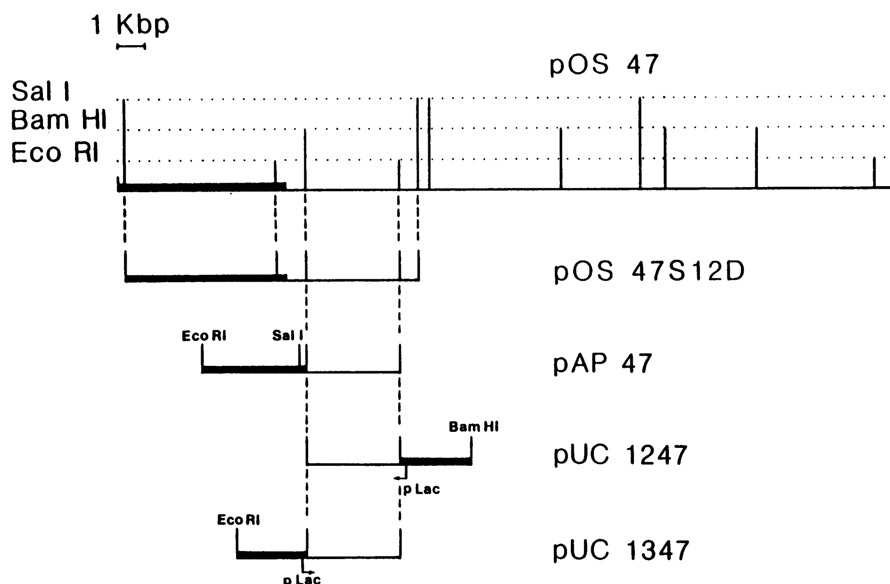


FIG. 2. Restriction map and subcloning of the pOS47 plasmid. *Eco*RI, *Bam*HI, and *Sal*I restriction sites are indicated. Solid bars show the DNA of the used vectors. In the case of pUC12 and pUC13 plasmids, the direction of the transcription initiated at the *lac* promoter (P Lac) is symbolized by an arrow. pOS47 DNA was partially hydrolyzed with *Sal*I and recircularized. Diadenosine tetraphosphatase specific activities were measured in crude extracts of IBPC111 transformed by the resulting deleted plasmids. pOS47S12D was characterized as the smallest deleted plasmid still overproducing this activity. pAP47, pUC1247, and pUC1347 were constructed by the insertion of the 3.5-kbp *Eco*RI-*Bam*HI fragment of pOS47S12D in the corresponding sites of pBR322, pUC12, and pUC13 vectors.

precipitate in 55% ammonium sulfate. The concentration was 3 to 4 mg of protein per ml. The titer was 75 nmol of diadenosine tetraphosphatase inactivated per mg of immunoglobulin.

Measurement of dinucleoside tetraphosphates in *E. coli* cells. Bacteria were grown in MOPS-glucose medium. During the exponential phase, 30-ml fractions were withdrawn and mixed with perchloric acid (10% [wt/wt] final concentration). The cellular extract was then centrifuged, neutralized with potassium carbonate, and purified on a DEAE-Sephadex column and on a boronate column, as previously described (A. Brevet, P. Plateau, M. Best-Belpomme, and S. Blanquet. J. Biol. Chem., in press). After this step, the sample was lyophilized and then dissolved in 100 μl of water. Ap_4N quantities were measured by bioluminescence using the Bioluminescence HS mixture and reagent from Boehringer in the presence of phosphodiesterase (P. Plateau, A. Brevet, and S. Blanquet, *Handbook of Chromatography*, in press). All the adenylylated dinucleoside tetraphosphates contributed to the luminescence. The sum of these nucleotides concentration was calculated, assuming a yield of ATP production from each dinucleoside tetraphosphate species equal to that from Ap_4A.

RESULTS

Selection of cosmid-overproducing diadenosine tetraphosphatase activity. Since no mutant strain affected in diadenosine tetraphosphatase activity was available, the search for a plasmid carrying the corresponding structural gene was based on the assumption that a strain harboring such a plasmid would overproduce this enzyme. A sensitive way to detect overproduction was to measure diadenosine tetraphosphatase activity in crude extracts. To minimize the number of clones to be assayed, a cosmid vector (pHC79) was used to construct a genomic library. In the case of pHC79, the average insert size was about 30 kilobase pairs (kbp), and thus the probability of obtaining a given gene among 500 clones should be higher than 0.99.

As described above, we adapted to pHC79 a strategy designed by Ish-Horowicz and Burke (15) for another cosmid vector. Two arms were prepared from the cosmid vector pHC79 (Fig. 1). Dephosphorylation of the *Eco*RI and *Sal*I termini prevented the formation of pHC79 multimers, thus eliminating background packageable molecules lacking inserts. Chromosomal DNA fragments to be inserted were dephosphorylated to reduce tandem insertions between the two arms. This treatment also simplified the chromosomal DNA preparation since DNA size fractionation was not needed. After ligation and in vitro packaging, 2×10^4 ampicillin-transducing particles per μg of *E. coli* chromosomal DNA were obtained. To check the efficiency of the gene bank, its ability to complement a Δ*lac* strain (IBPC111) was tested. Among 500 ampicillin-resistant transductants, 5 clones appeared blue on 5-bromo-4-chloro-3-indolyl-β-D-galacto-pyranoside plates.

A total of 500 clones were screened by the method described above. Parallel [^{14}C]Ile incorporation measurements allowed us to standardize the enzyme activities in the extracts in a rapid and reliable way. For each set of 25 assays, the standard deviation appeared to be less than 30% of the mean specific activity value. This enabled us to detect, among the 500 clones tested, one clone which displayed a specific activity that was three- to fourfold higher than that of the host strain. This clone was shown to harbor a hybrid cosmid, called pOS47, carrying an insert of 24 kbp (Fig. 2). To demonstrate that overproduction was dependent on the presence of pOS47, it was verified that another strain (HB101) transformed by pOS47-purified DNA also overproduced diadenosine tetraphosphatase activity.

Restriction enzyme analysis, subcloning of the pOS47 cosmid, and identification of the *apaH* structural gene. *Sal*I, *Bam*HI, and *Eco*RI restriction sites were mapped on the

TABLE 2. Overproduction of diadenosine tetraphosphatase

Strains	Overproduction factor[a]
IBPC111	1
IBPC111-pHC79	0.7 ± 0.2
IBPC111-pBR322	0.8 ± 0.1
IBPC111-pUC12	0.9 ± 0.1
IBPC111-pOS47	3.0 ± 1.0
IBPC111-pOS47S12D	12.0 ± 2.0
IBPC111-pAP47	6.0 ± 2.0
IBPC111-pUC1247	31.0 ± 4.0
IBPC111-pUC1247B	0.9 ± 0.1
IBPC111-pUC1247PBD	30.0 ± 4.0

[a] Diadenosine tetraphosphatase specific activities were measured in crude extracts obtained by ultrasonic disintegration of cells grown at 37°C in LB medium (supplemented with 50 μg of ampicillin per ml when needed) and harvested in midexponential phase. Overproduction factor is the ratio of the specific activity of each strain over that of IBPC111 (10 U/mg of protein; protein concentration was determined by the biuret method).

cosmid DNA (Fig. 2). pOS47 DNA was submitted to partial *Sal*I digestion. After circularization and transformation of strain IBPC111, the resulting deleted plasmids were characterized by restriction mapping. Specific activities of diadenosine tetraphosphatases were determined in parallel in crude extracts. The smallest deleted plasmid which still overproduced diadenosine tetraphosphatase activity was chosen for further subcloning. This plasmid, called pOS47S12D, was derived from pOS47 by a 19.5-kbp *Sal*I deletion, and carried a 4.5-kbp chromosomal insert (Fig. 2). A. 3.5-kbp *Eco*RI-*Bam*HI fragment prepared from this plasmid was cloned between the corresponding sites of pBR322, pUC12, and pUC13 vectors to give, respectively, pAP47, pUC1247, and pUC1347 (Fig. 2). Levels of diadenosine tetraphosphatase activity were measured in crude extracts of IBPC111 transformed by pAP47 and of JM101 (*lacI*^{q}) transformed by pUC1247 and pUC1347 (Table 2; see Fig. 4). The results demonstrate that the *Eco*RI-*Bam*HI fragment of pOS47 carried the gene stimulating diadenosine tetraphosphatase activity.

To identify the proteins encoded by the *Eco*RI-*Bam*HI DNA fragment, maxicell experiments were carried out. Plasmid pUC1247 directed the synthesis of two polypeptides, with the major polypeptide corresponding to the pUC12-encoded β-lactamase (Fig. 3). A minor band, comigrating with purified diadenosine tetraphosphatase (apparent M_r of 32,000 [32K]) was expressed from pUC1247 but not from pUC12. Identification of this product was achieved with antibodies directed against homogenous diadenosine tetraphosphatase. As shown in Fig. 3 (lane c), the 32K polypeptide could be immunoprecipitated. This result unambiguously demonstrates that the 3.5-kbp *Eco*RI-*Bam*HI fragment of pOS47 carries the structural gene for diadenosine tetraphosphatase. This gene is called *apaH*.

Transcription of the *apaH* gene. In plasmids pUC12 and pUC13, transcription of cloned genes from the IPTG inducible *lac* promoter depends on their polarity of insertion. The results presented in Fig. 4 indicated that addition of isopropyl-β-D-thiogalactopyranoside (IPTG) resulted in an eightfold increase of *apaH* expression in JM101(pUC1347), whereas it had no effect on JM101(pUC1247). Therefore, transcription of the *apaH* gene should occur from *Bam*HI to *Eco*RI.

To map more precisely the *apaH* gene, the following experiments were carried out. Plasmid pUC1247 was cut by *Bst*EII (Fig. 4) and circularized after the 3'-recessed termini was filled in to give plasmid pUC1247B. This five-nucleotide insertion at the unique *Bst*EII site resulted in the complete loss of diadenosine tetraphosphatase overproduction (Table 2). On the contrary, deletion of the 1.5-kbp *Pvu*II-*Bam*HI fragment of pUC1247 (plasmid pUC1247PBD in Fig. 4) did not affect diadenosine tetraphosphatase overproduction (Table 2). This result indicates that the 2-kbp *Eco*RI-*Pvu*II region contains the structural part of *apaH*. In addition, since the *apaH* structural gene should be about 1 kbp long and the distance between *Eco*RI and *Bst*EII is only 0.5 kbp, it can be concluded from the result with plasmid pUC1247B

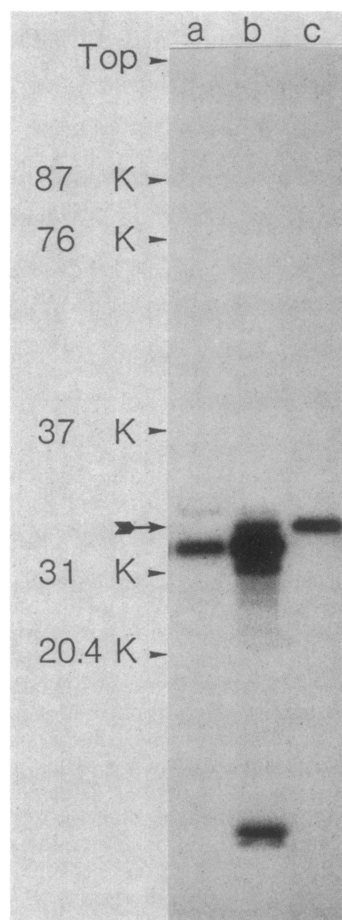
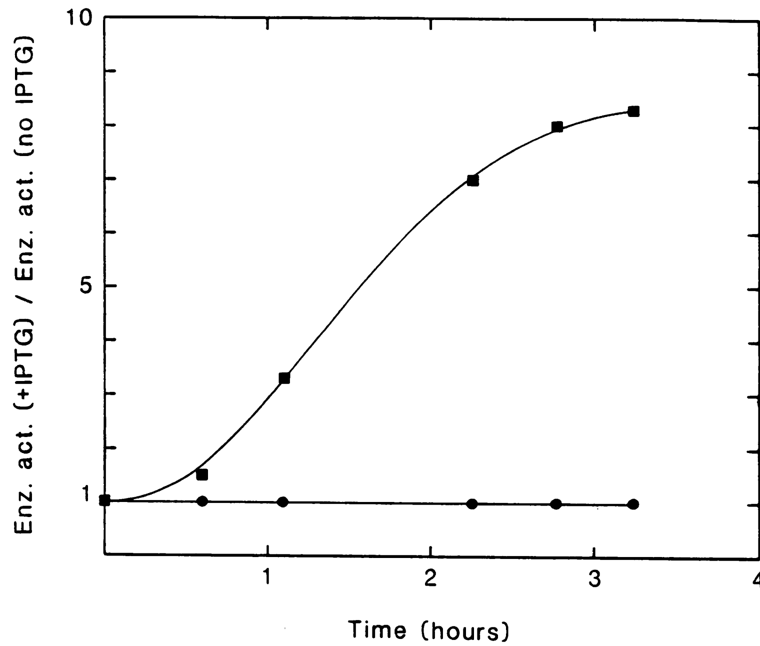


FIG. 3. Identification of the proteins expressed from plasmid pUC1247. CSR603 cells transformed by plasmids pUC12 or pUC1247 were UV irradiated and pulse-labeled with [^{35}S]methionine (maxicells), before preparation and SDS-PAGE analysis of crude extracts (18). The position of molecular weight markers are indicated by arrowheads beside the fluorogram. Migration of purified unlabeled diadenosine tetraphosphatase is shown by a horizontal arrow. (Lane a) Products expressed from pUC12. The major band corresponds to the mature β-lactamase encoded by the *bla* gene carried by pUC12. (Lane b) Products expressed from pUC1247. The major band corresponds to the β-lactamase. The upper band comigrates with purified diadenosine tetraphosphatase. The band which migrates slightly faster than β-lactamase may be a degradation product since it was not reproducibly observed. An additional band with an apparent M_r of 14K, also encoded by the chromosomal insert of pUC1247, is observed. (Lane c) The crude extract of CSR603(pUC1247) was treated with antibodies directed against diadenosine tetraphosphatase and protein A-Sepharose. The only immunoprecipitated product corresponds to the band comigrating with homogeneous diadenosine tetraphosphatase.



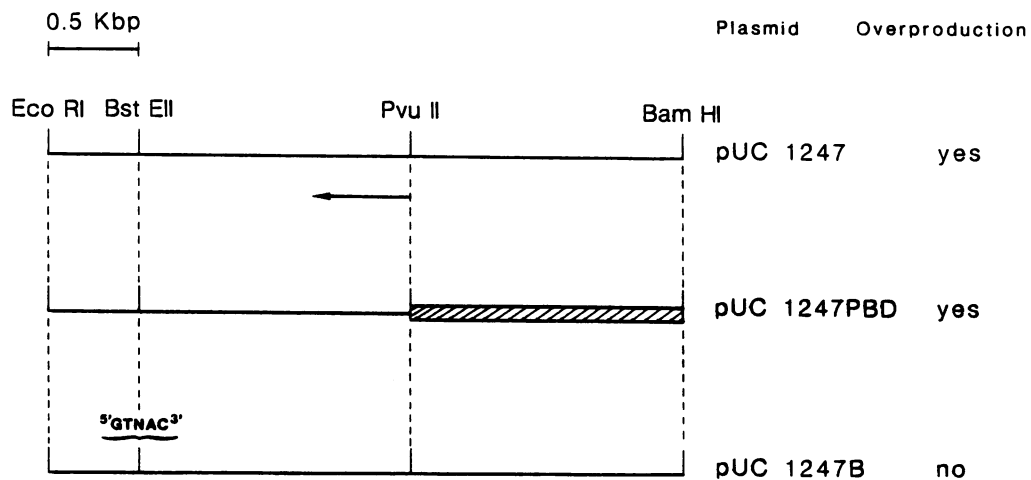


FIG. 4. Transcription and localization of the *apaH* gene. (A) JM101(pUC1247) and -(pUC1347) strains were grown at 37°C up to an optical density at 650 nm of 0.15 in LB medium supplemented with 50 μg of ampicillin per ml. To one half of each culture, IPTG was added at a final concentration of 5 mM (time zero on the figure). Fractions (10 ml) were withdrawn at the indicated times, and enzyme specific activities (Enz. act.) were measured in crude extracts obtained by ultrasonic disintegration. Results are plotted as the ratio of the specific activity from cells incubated in the presence of IPTG over that of cells grown in the absence versus the time course of incubation; Symbols: ●, JM101(pUC1247); ■, JM101(pUC1347). In the presence or in the absence of IPTG, the generation time was 26 min for both strains. Specific enzyme activities at the end of the experiment were 300 U/mg of protein for JM101(pUC1247) (30-fold overproduction) in the presence or absence of IPTG; 100 and 800 U/mg for JM101(pUC1347) (10- and 80-fold overproduction) in the absence and presence of IPTG, respectively. (B) Partial restriction map of the *E. coli* chromosomal insert of pUC1247 (Top). Plasmid pUC1247PBD was derived from pUC1247 after deletion of the *Pvu*II-*Bam*HI fragment (hatched box). Five nucleotides were inserted into the *Bst*EII site of pUC1247 by filling in the 5′ protruding termini to give pUC1247B (bottom). Direction of *apaH* transcription is indicated by the horizontal arrow.

that the *Bst*EII site is internal to the *apaH* coding region. The 5′ end of the *apaH* structural gene therefore should be located 0.5 to 1 kbp upstream from this *Bst*EII site.

Effect of diadenosine tetraphosphatase overproduction on dinucleoside tetraphosphate concentrations in *E. coli* cells. To establish that the in vitro-characterized diadenosine tetraphosphatase is well involved in dinucleoside polyphosphate catabolism in vivo, Ap_4N concentrations were measured in cultures of strain IBPC111 carrying either pUC12 or pUC1247. In the case of IBPC111(pUC1247) (generation time, 100 min), which overproduced diadenosine tetraphosphatase 30-fold, the intracellular concentration of dinucleo-

side tetraphosphates was equal to 0.14 μM. In the control strain IBPC111(pUC12) (generation time, 90 min), the Ap_4N concentration was 1.4 μM.

DISCUSSION

Construction of a cosmid library allowed us to isolate an *E. coli* clone (cosmid pOS47) which overproduced diadenosine tetraphosphatase activity. It was demonstrated that the gene (*apaH*) coding for diadenosine tetraphosphatase was carried by a 3.5-kbp *Eco*RI-*Bam*HI fragment, derived from the pOS47 cosmid.

The IPTG dependence of the diadenosine tetraphosphatase overproduction in JM101(pUC1347) showed that in this plasmid, expression of the *apaH* gene was under the control of the *lac* promoter. Therefore, transcription of *apaH* should occur from *Bam*HI to *Eco*RI. In the absence of IPTG, pUC1347 as well as pUC1247 cause overproduction of the enzyme (Table 2 and legend to Fig. 4). There is, however, some discrepancy between the two strains which might reflect different plasmid copy numbers. This overproduction suggests that the *Eco*RI-*Bam*HI fragment also carries the *apaH* transcription promoter. This promoter should be located to the left of the *Pvu*II site (Fig. 4) since deletion of the *Pvu*II-*Bam*HI fragment did not modify the overproduction level.

Finally, it is shown that an increase of the diadenosine tetraphosphatase concentration in *E. coli* cells is accompanied by a drop of the Ap_4N concentration. This observation establishes that the Ap_4N-catabolizing properties of the enzyme described in vitro are valid in vivo.

The cloning of the *apaH* gene and the availability of *E. coli* strains affected in their Ap_4N concentration opens a new way to search for the physiological role of these dinucleotides. It already can be noted from the results that the reduced level of Ap_4N caused by a 30-fold overproduction of diadenosine tetraphosphatase does not appear to affect the bacterial growth rate.

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LITERATURE CITED

1. **Barnes, L. D., and C. A. Culver.** 1982. Isolation and characterization of diadenosine 5', 5'''-P^1,P^4 tetraphosphate pyrophosphohydrolase from *Physarum polycephalum*. Biochemistry **21**:6123–6128.
2. **Blanquet, S., P. Plateau, and A. Brevet.** 1983. The role of zinc in 5',5'-diadenosine tetraphosphate production by aminoacyl-transfer RNA synthetases. Mol. Cell. Biochem. **52**:3–11.
3. **Bochner, B. R., P. C. Lee, S. W. Wilson, C. W. Cutler, and B. N. Ames.** 1984. AppppA and related adenylylated nucleotides are synthesized as a consequence of oxidation stress. Cell **37**:225–232.
4. **Bolivar, F., R. C. Rodriguez, P. J. Greene, M. C. Betlach, H. L. Heyneker, and J. Boyer.** 1977. Construction and characterization of new cloning vehicles. II. A multipurpose cloning system. Gene **2**:95–113.
5. **Boyer, H. W., and D. Roulland-Dussoix.** 1969. A complementation analysis of the restriction and modification of DNA in *Escherichia coli*. J. Mol. Biol. **41**:459–472.
6. **Brevet, A., P. Plateau, B. Cirakoglu, J. P. Pailliez, and S. Blanquet.** 1982. Zinc-dependent synthesis of 5',5'-diadenosine tetraphosphate by sheep liver lysyl- and phenylalanyl-tRNA synthetases. J. Biol. Chem. **257**:14613–14615.
7. **Cameselle, J. C., M. J. Costas, M. A. G. Sillero, and A. Sillero.** 1984. Two low Km hydrolytic activities on dinucleoside 5',5'''-P^1,P^4 tetraphosphates in rat liver. J. Biol. Chem. **259**:2879–2885.
8. **Goerlich, O., R. Foeckler, and E. Holler.** 1982. Mechanism of synthesis of adenosine (5') tetraphospho (5') adenosine (AppppA) by aminoacyl-tRNA synthetases. Eur. J. Biochem. **126**:135–142.
9. **Grummt, F.** 1983. Diadenosine tetraphosphate (Ap_4A): a putative chemical messenger of cell proliferation control and inducer of DNA replication. Plant Mol. Biol. **2**:41–44.
10. **Guranowski, A., H. Jakubowski, and E. Holler.** 1983. Catabolism of diadenosine 5', 5'''-P^1, P^4 tetraphosphate in prokaryotes. J. Biol. Chem. **258**:14784–14789.
11. **Harris-Warrick, R. M., Y. Elkana, S. D. Ehrlich, and L. Lederberg.** 1976. Electrophoretic separation of *Bacillus subtilis* genes. Proc. Natl. Acad. Sci. USA **72**:2207–2211.
12. **Hohn, B.** 1979. *In vitro* packaging of λ and cosmid DNA. Methods Enzymol. **68**:299–309.
13. **Hohn, B., and J. Collins.** 1980. A small cosmid for efficient cloning of large DNA fragments. Gene **11**:291–298.
14. **Höhn, M., W. Albert, and F. Grummt.** 1982. Diadenosine tetraphosphate hydrolase from mouse liver. J. Biol. Chem. **257**:3003–3007.
15. **Ish-Horowicz, D., and J. F. Burke.** 1981. Rapid and efficient cosmid cloning. Nucleic Acids Res. **9**:2989–2998.
16. **Jakubowski, H.** 1983. Synthesis of diadenosine 5',5'''-P^1,P^4 tetraphosphate and related compounds by plant (*Lupinus luteus*) seryl-tRNA and phenylalanyl-tRNA synthetases. Acta Biochim. Polon. **30**:51–69.
17. **Jakubowski, H., and A. Guranowski.** 1983. Enzymes hydrolyzing ApppA and/or AppppA in higher plants. J. Biol. Chem. **258**:9982–9989.
18. **Laemmli, U. K.** 1970. Cleavage of structural proteins during the assembly of the head of bacteriophage T4. Nature (London) **227**:180–182.
19. **Maniatis, T., E. F. Fritsch, and J. Sambrook.** 1982. Molecular cloning. A laboratory manual. Cold Spring Harbor Laboratory. Cold Spring Harbor, N.Y.
20. **Messing, J.** 1983. New M13 vectors for cloning. Methods Enzymol. **101**:20–78.
21. **Miller, J. H.** 1972. Experiments in molecular genetics. Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y.
22. **Neidhart, F. C., P. L. Bloch, and D. F. Smith.** 1974. Culture medium for enterobacteria. J. Bacteriol. **119**:736–747.
23. **Ogilvie, A., and W. Antl.** 1983. Diadenosine tetraphosphatase from human leukemia cells. J. Biol. Chem. **258**:4105–4109.
24. **Plateau, P., M. Fromant, A. Brevet, A. Gesquière, and S. Blanquet.** 1985. Catabolism of bis (5'-nucleosidyl) oligophosphates in *Escherichia coli*: metal requirement and substrate specificity of homogeneous diadenosine 5',5'''-P^1,P^4 tetraphosphate pyrophosphohydrolase. Biochemistry. **24**:914–922.
25. **Plateau, P., J. F. Mayaux, and S. Blanquet.** 1981. Zinc (II)-dependent synthesis of diadenosine 5', 5'''-P^1,P^4 tetraphosphate by *Escherichia coli* and yeast phenylalanyl-transfer ribonucleic acid synthetases. Biochemistry **20**:4654–4662.
26. **Rapaport, E., and P. C. Zamecnik.** 1976. Presence of diadenosine 5', 5'''-P^1,P^4 tetraphosphate (Ap_4A) in mammalian cells in levels varying widely with proliferative activity of the tissue: a possible positive "pleiotypic activator". Proc. Natl. Acad. Sci. USA **73**:3984–3988.
27. **Sancar, A., R. P. Wharton, S. Seltzer, B. M. Kacinski, N. D. Clarke, and W. D. Rupp.** 1981. Identification of the *uvrA* gene product. J. Mol. Biol. **148**:45–62.
28. **Springer, M., M. Trudel, M. Graffe, J. A. Plumbridge, G. Fayat, J. F. Mayaux, C. Sacerdot, S. Blanquet, and M. Grunberg-Manago.** 1983. *Escherichia coli* phenylalanyl-tRNA synthetase operon is controlled by attenuation *in vivo*. J. Mol. Biol. **171**:263–279.
29. **Vieira, J., and J. Messing.** 1982. The pUC plasmids, an M13mp7 derived system for insertion mutagenesis and sequencing with synthetic universal primers. Gene **19**:259–268.
30. **Zamecnik, P.** 1983. Diadenosine 5', 5'''-P^1, P^4 tetraphosphate

(Ap_4A): its role in cellular metabolism. Anal. Biochem. **134**:1–10.

31. **Zamecnik, P. C., and M. L. Stephenson.** 1969. Nucleoside pyrophosphate compounds related to the first step in protein synthesis, p. 276–291. *In* H. M. Kalckar, H. Klenow, G. Munch-Petersen, M. Ottesen, and J. M. Thuysen, (ed.), The role of nucleotides for the function and conformation of enzymes. Alfred Benzon Symposium I, Munksgaard Pub., Copenhagen.
32. **Zamecnik, P. C., M. L. Stephenson, C. M. Janeway, and K. Randerath.** 1966. Enzymatic synthesis of diadenosine tetraphosphate and diadenosine triphosphate with a purified lysyl-sRNA synthetase. Biochem. Biophys. Res. Commun. **24**:91–97.