Isolation and Mapping of Polynucleotide Phosphorylase Mutants of Escherichia coli

ALBEY M. REINER

Three strains of Escherichia coli with altered polynucleotide phosphorylase, Q7, Q13, and Q27, were isolated by screening clones from heavily mutagenized cultures for low levels of the enzyme. The three mutations were found to cotransduce with argC and asp, and the pnp locus which they define was mapped with respect to these loci. An explanation for the nonreciprocal cotransduction frequencies observed with asp is provided by the demonstration of an unlinked asp-suppressing locus.

Polynucleotide phosphorylase (PNPase) catalyzes the phosphorolysis of ribonucleic acid (RNA) to nucleoside diphosphates, the synthesis of polynucleotides from nucleoside diphosphates, and an exchange reaction between the β-phosphate of the diphosphate and inorganic phosphate (4, 7). The physiological function of the enzyme is unknown. Random ordering of nucleotides in the polymerization reaction and the relatively weak affinity of PNPase for diphosphates make it unlikely that polymerization is significant in vivo. Because of the energy conservation of its phosphorolysis reaction and the implication of diphosphates as the breakdown products of messenger RNA (mRNA), PNPase is a good candidate for involvement in mRNA breakdown (15). To pursue this problem further, a search for PNPase mutants of Escherichia coli was undertaken. Three strains with altered PNPase were isolated by screening 950 heavily mutagenized clones for their PNPase level.

In achieving a mutation frequency of 3 in 950 for PNPase, many other mutations were induced in each strain. To characterize the PNPase mutations and look for possible physiological effects, it was desirable to separate the PNPase lesions from the others present in the original isolates. Transduction by phage P1 provides a means to transfer each of the PNPase lesions into a genetically characterized strain and thus to construct a family of strains which differ only in a small region surrounding the PNPase locus. Since PNPase is a nonselectable trait, it was first necessary to know its location on the chromosome so that it might be transferred by cotransduction with a neighboring selectable marker. This paper describes the isolation of the three PNPase mutants and the mapping of the pnp locus. In the accompanying paper (13) the mutations are characterized in nearly isogenic strains.

MATERIALS AND METHODS

Parental strain. The parental strain A19 is a ribonucleic acid (RNA)–lysogen, HfrH, λ lysogen with an unidentified growth requirement. It was isolated by Gesteland (3) by nitrosoguanidine mutagenesis of AB301 (Met−, λ lysogen, HfrH). The unidentified defect is a trace requirement, since its removal by transduction results in colonies which support a substantial surrounding halo of growth of A19 (14).

Bacterial growth. Bacteria were grown in yeast-trypotone (YT) broth containing 5 g of NaCl, 8 g of tryptone (Difco), and 5 g of yeast extract per liter. Mineral medium for selection plates contained (per liter): 5 g of NaCl, 1 g of NH₄Cl, 0.25 g of MgSO₄, 0.15 g of K₂HPO₄, 2 g of glucose, 75 ml of 1 M tris(hydroxymethyl)aminomethane (Tris), pH 7.6 at 37°C, and a trace of thiamine. Amino acids other than arginine and aspartate were added by spreading 0.1 ml of 1% l-amino acid per plate. L-Aspartate (250 mg/liter) and L-arginine (15 mg/liter) were added to plates before pouring. Materials used as carbon sources were added to a final concentration of 20 mM.

Mutagenesis. Bacteria cultures were grown over-
night in YT at 37 C, and 10-ml amounts were centrifuged. The cells were washed once and resuspended in 0.5 ml of 0.2 M sodium acetate buffer (pH 5.0). A 0.1-ml amount of nitrosoguanidine (4 mg/ml) was added, and the mixture was incubated at 37 C without shaking for 3 hr. Bacteria were then diluted 1:50 into fresh YT, separated into 50 tubes, shaken overnight at 25 C to allow segregation, and plated.

**Screening for PNPase mutants.** Following mutagenesis and segregation, individual colonies were spotted onto duplicate YT plates and were grown overnight at 30 C. From one plate, each spot was suspended in 150 µl of 0.02 M Tris (pH 8.0) plus 10⁻⁴ M ethylenediaminetetraacetate (EDTA), with one drop of toluene per tube. The tubes were incubated for 60 min at 43 C, and the contents were then assayed for PNPase by inorganic ³²P-adenosine diphosphate (ADP) exchange as follows. A 300-µl liter amount of solution was added to the cell suspension to achieve a final concentration of 100 mM Tris (pH 8.0), 2.5 mM ADP, and 2.5 mM sodium phosphate containing 10,000 to 100,000 counts/min of ³²P. (carrier-free, Tracerlab, Waltham, Mass.), 5 mM MgCl₂, and 0.5 mM EDTA. The reaction mixture was incubated for 120 min at 43 C and the reaction was stopped by adding 0.7 ml of cold 7% perchloric acid containing 20 mg of Norit. After 10 min at 0 C, the suspension was filtered on a glass-fiber filter. The filter was washed with 18 ml of water containing dissolved inorganic phosphate and was then glued Norit-side down to a planchet and counted. With this procedure, 100 clones/day could be screened conveniently.

**PNPase assays in cell extracts.** Cultures were harvested in late log phase (optical density of 2.0 to 2.5 in YT at 37 C). Cells were washed in 0.02 M Tris (pH 7.8), pelleted, frozen and ground with 2.5 g of alumina per g of frozen cells. Alumina and debris were removed by centrifugation, and the supernatant fluid was dialyzed two times against 500 volumes of 0.02 M Tris (pH 7.8). Extracts contained 75 to 150 mg of protein/ml (biuret), and 5-µl liter amounts of extract were used in 100-µl liter reaction mixtures.

The exchange reaction mixture was at the concentration of the screening assay. The phosphorolysis reaction was the same except that ADP was replaced by 0.5 mg of polyadenylic acid (Miles Laboratories, Elkhart, Ind.) per ml, and the sodium phosphate concentration was 10 mM. The polymerization reaction mixture was 75 mM Tris (pH 8.0), 5 mM MgCl₂, and 5 mM ADP containing 20,000 counts/min of ³¹C-ADP (50 mc/mmol; Schwarz BioResearch, Inc., Orangeburg, N.Y.). The exchange and phosphorolysis reactions were stopped after 10 min at 37 C by adding 0.5 ml of cold 5% perchloric acid containing 20 mg of Norit; the Norit was filtered, washed, and counted as described above. The polymerization reaction was stopped after 10 min by adding 0.5 ml of cold 5% perchloric acid. The mixture stood for 10 min at 0 C and was then filtered on DA filters (Millipore Corp., Bedford, Mass.), washed three times with 2.5 ml of 1% cold perchloric acid, dried, and counted on planchets.

**Scoring the pnp alleles.** PNPase can be conveniently assayed in toluene-treated cells by either the exchange or the polymerization reaction; polymerization was routinely used in scoring. To distinguish either pnp-7 or pnp-13 (the defects originally isolated in Q7 and Q13, respectively) from the wild-type allele, colonies were spotted on YT plates, grown overnight at 37 C, suspended in 100 µl of 0.02 M Tris (pH 8.0), and made porous by incubating at 37 C for 10 min with 10 µl of toluene. Samples (50 µl) were assayed in 100-µl liter reaction mixtures, at the conditions of the polymerization assay in cell extracts. In a typical scoring, 50 pnp-7 or pnp-13 clones incorporated 10 to 30 counts/min, and 50 wild-type clones incorporated 110 to 170 counts/min. To distinguish pnp-27 from the wild-type allele, colonies were grown overnight at 43 C in YT liquid containing 0.2% glucose. A 300-µl liter amount was centrifuged and the cells were washed once, suspended in 50 µl of 0.02 M Tris (pH 8.0), and assayed as above.

The rationale for these assays will be apparent when the characterization of the altered PNPases is presented (13). Briefly, the pnp-7 enzyme has undetectable polymerization activity and the pnp-13 enzyme is virtually inactive because Mn⁺⁺ is not present. The pnp-27 enzyme is temperature-sensitive and in nongrowing cells is inactivated at 43 C if conditions are acidic. Overnight cultures grown on YT plus glucose reach pH 6 or less and inactivate the pnp-27 enzyme but not the wild type.

**Bacterial crosses.** Log-phase cells grown at 37 C in YT plus 0.2% glucose were mixed to final concentrations of 5 X 10⁹ males per ml plus 5 X 10⁸ females per ml, and were incubated at 37 C with large surface exposure but without shaking. For interrupted mating, this mixture was diluted 1:100 after 4 min into fresh YT plus 0.2% glucose and incubated as above. At appropriate times, 1-ml samples were removed and vortexed for 60 sec at top speed on a Super-Mixer no. 1290 (Lab-line Instruments, Melrose Park, Ill.). 0.1-ml portions were then plated.

**P1 Transduction.** For preparation of P1 stocks, 2 X 10⁸ P1 grown on AB301, the parent strain of A19, were added to 2 X 10⁶ bacteria in YT plus 10 mM CaCl₂, incubated for 15 min at 37 C, and plated onto YT plates from 2.5 ml of YT-soft agar. Plates and soft agar contained 10 mM CaCl₂. After 6 hr at 37 C, the plates were scraped, the soft agar was removed by centrifugation, and the supernatant liquid was chloroformed. Yields were 4 X 10⁹ to 8 X 10⁹ phage per plate, titered on Shigella. For transduction 2 X 10⁹ recipient bacteria were mixed with 10⁶ P1 in 0.2 ml of YT plus 10 mM CaCl₂, incubated at 37 C for 10 min, and spread onto selection plates.

**RESULTS**

**Isolation of PNPase mutants.** Since no physiological function for PNPase was known, no selection procedure for mutants was possible. A rapid, specific assay for PNPase on a small quantity of cells was required to enable the screening of a large number of mutagenized...
strains. In the screening procedure devised, individual clones were suspended in a small volume of buffer, made porous by toluene, and assayed for PNPase level (see Materials and Methods).

Three strains exhibiting low \(^3^P\)-ADP exchange activity in tolenuenized cells were detected. Q7 and Q13 were isolated in Walter Gilbert's laboratory on the same day by the author and Diane Vargo. Several months later, Dr. Gilbert resumed the mutant search, using a screening pool enriched with temperature-sensitive strains obtained by pretesting a larger mutagenized stock for clones which grew on YT at 25 C but not at 43 C. Q27 was one of these temperature-sensitive strains. To confirm that the three strains were mutant in PNPase, Q7, Q13, and Q27 were assayed for PNPase under standard conditions by polymerization, phosphorolysis, and exchange assays in cell extracts. Q7 and Q13 showed <10% wild-type activity, and Q27 could be heat-inactivated to this level in 10 min at 48 C (the wild-type enzyme was stable at 38 C). Experiments in which mutant and wild-type extracts were mixed showed that inhibitors were not the cause of the reduced activities. The lesions are designated pnp-7, pnp-13, and pnp-27, respectively.

The PNPase mutants have been used in other laboratories for studies on RNA breakdown (1, 8–10) and to provide conditions of reduced nuclease activity (5, 16, 17). In addition, Thang, Buchanan, and Grunberg-Manago (19) and Hsieh and Buchanan (6) have reported on the PNPase in Q13. My characterization of the pnp-7, pnp-13, and pnp-27 mutations is presented in the accompanying paper (13).

Phenotypic properties of the mutant strains. Additional mutations were apparent in these strains. Q7 reverted to ribonuclease T\(^1\) and lost the A19 trace requirement. Q13 retains the A19 defects and in addition requires tyrosine. Kivity-Vogel and Elson (8) reported that its \(\beta\)-galactosidase is unstable. Natori and Mizuno (9) reported growth of Q13 on a glucose-mineral medium supplemented by methionine, tyrosine, leucine, and tryptophan, but in my medium no combination of amino acids without a trace addition of yeast extract supported growth. Q27 grew in liquid YT at 43 C but not on YT plates at 43 C. By transduction, the temperature sensitivity of the strain was shown to be unrelated to the pnp-27 defect (13). Q27 has lost the Hfr properties of A19; it does not donate markers in conjugation and is not sensitive to R17 male-specific phage.

An artifact involving the exchange reaction of Q13 was observed. Although dialyzed Q13 extracts catalyze ADP-stimulated \(^3^P\)-incorporation into Norit-adsorbing material at <10% of the wild-type level, undialyzed Q13 extracts catalyze incorporation at 100% of the wild-type level. More than 95% of the Q13 activity which disappears upon dialysis reappears with the addition of boiled cell extract to the reaction mixture. In contrast, extracts from the parental strain A19 lose no activity upon exhaustive dialysis, and neither Q7 nor A19 gain any activity from boiled cell extract. The active component was purified from boiled extract by adsorption to and desorption from Norit and by fractionation on a Biogel P2 and a diethylaminoethyl (DEAE) cellulose column. Following electrophoresis and measurement of its absorption spectrum, the component was tentatively identified as coenzyme A. Chromatographically pure coenzyme A (PL Biochemicals, Milwaukee, Wis.) was run on a DEAE column, and the major peak, which contained 80% of the ultraviolet-absorbing material and eluted at the position of the component purified from cell extract, was found to be highly active. Although boiled cell extract stimulated only Q13 extracts, purified coenzyme A stimulated Q7 and A19 extracts as well. The radioactive product of the reaction stimulated by either coenzyme A or boiled extract was identified as adenosine triphosphate [by Dowex-1 column chromatography and isopropanol-1% ammonium sulfite (2:1) paper chromatography]. The observed reactions are thus similar to those of the succinyl coenzyme A synthase system (12), and a mutation involving one of these enzymes, unrelated to PNPase, may have occurred in Q13.

Mapping of the pnp locus near argG and asp. Preliminary crosses of Q7 with a multiply marked F\(^-\) strain showed a high linkage of pnp-7 with argG (Fig. 1). When Q7 or Q13 was used as Hfr donor in conjugation, it was difficult to transfer pnp or earlier loci by selecting on markers other than argG. This difficulty is not due to zygotic induction of \(\lambda\), and it is a property of the parental line of the PNPase mutants (14).] Transducing AB1302 (argG, etc.; see Table 1 for description of strains) to Arg\(^+\) by P1(Q7) (P1 phage grown on bacterial strain Q7) and scoring for pnp-7 confirmed this linkage, showing a cotransduction frequency of 75 to 80% for argG and pnp-7. Since asp was known from mating experiments to map near argG (R. Lavellé, personal communication), PA3403 (asp, etc.) was transduced to Asp\(^+\) by P1(Q7). A cotransduction frequency of 30 to 35% was obtained for asp and pnp-7. Transducing with P1(Q13) and P1(Q27), these same approximate linkages of pnp to argG and asp were obtained. The three mutations were assumed to map at a single structural gene for PNPase, which was designated pnp.
Ordering of \textit{asp}, \textit{argG}, and \textit{pnp}. Two positions of \textit{pnp} are consistent with the transduction data: between \textit{asp} and \textit{argG} or on the side of \textit{argG}, opposite from \textit{asp}. To order uniquely the three genes, an \textit{argG} strain was constructed by crossing AT12-55 (Hfr AB312, \textit{argG}, etc.) with PA3403L (\textit{asp ilv}, etc.), selecting for \textit{Ilv}+, and scoring the recombinants for \textit{Asp} and \textit{Arg}. Of the 440 recombinants, 6 were \textit{Asp}– \textit{Arg}–, and one such strain, AA1, was selected for further use. AA1 was transduced with PI(Q13), either selecting for \textit{Asp}+ and scoring for \textit{pnp} and \textit{argG} or selecting for \textit{Arg}+ and scoring for \textit{pnp}. The results from 200 transductants (Table 2 and Fig. 2) uniquely determine the order \textit{asp argG pnp}. (AA1 is more likely to receive \textit{argG}+ alone than \textit{pnp}–13 alone in \textit{Asp}+ selection, showing that \textit{argG} is the nearer locus to \textit{asp}.)

Scoring for \textit{asp} was unreliable, because the \textit{Asp}– colonies grew more poorly than the \textit{Asp}+ at all concentrations of aspartate tripeptide, and often were lost. This did not affect the above cotransduction frequencies, however, since linkage of \textit{pnp} and \textit{argG} determined separately for 50 \textit{Asp}+ and 50 \textit{Asp}– transductants was 76 and 78%, respectively. High concentrations of aspartate partially satisfy the arginine requirement in some \textit{argG} strains. At 2 mM exogenous aspartate, the \textit{asp} requirement is satisfied whereas the \textit{argG} requirements remain strict. (The \textit{argG} enzyme catalyzes the synthesis of argininosuccinate from aspartate and citruline, and increased aspartate concentrations presumably enable the mutant enzyme to function partially.)

Ordering of \textit{asp}, \textit{argG}, \textit{pnp}, and \textit{str}. To verify the orientation of \textit{asp argG pnp} on the chromosome, \textit{asp} and \textit{argG} were ordered with respect to \textit{str} by crossing AT12-55 (\textit{argG Sm} SmR T6X, etc.) \times PA3403L (\textit{asp ilv Sm} SmR T6X, etc.), selecting for \textit{Ilv}+ \textit{Asp}+ \textit{Arg}+ recombinants, counterselecting

\begin{table}
\centering
\caption{Strains used$^a$}
\begin{tabular}{lllll}
\hline
Strain & Sex & Markers & Source & \\
\hline
A19 & HfrH & RNAse I+ Met+, unidentified trace requirement, (\textlambda{}) & Gesteland & \\
Q7 & HfrH & pnp-7 Met+ (\textlambda{}) & Mutagenesis of A19 & \\
Q13 & HfrH & pnp-13 RNAse I+ Met+ Tyr+, unidentified trace requirement, (\textlambda{}) & Mutagenesis of A19 & \\
Q27 & ? & pnp-27 RNAse I+, temperature-sensitive growth (see text), (\textlambda{}) & Mutagenesis of A19 & \\
AB301 & HfrH & Met+ (\textlambda{}) & Adelberg & \\
AB1302 & F– & thr leu argG thi lac gal mal xyl mtl SmR & Gorini & \\
PA3403 & F– & his asp lac gal mal xyl mtl SmR T6X & Lavallé & \\
PA684 & Hfr AB312 & ilv Arg+ Pur– xyl & Adelberg & \\
PA3403L & F– & ilv asp Pur– lac gal xyl T6X & AB684 \times PA3403 & \\
AT12-55 & Hfr AB312 & thr leu argG thi SmR & Lavallé & \\
AA1 & F– & argG asp Pur– lac gal xyl SmR T6X & AT12-55 \times PA3403L & \\
AT2446 & HfrH & & Taylor & \\
\hline
\end{tabular}
\footnote{Abbreviations: \textit{arg}, \textit{asp}, \textit{his}, \textit{ilv}, \textit{leu}, \textit{met}, \textit{Pur}–, \textit{thi}, \textit{thr}, and \textit{Tyr}– denote inability to grow without arginine, aspartate, histidine, isoleucine and valine, leucine, methionine, adenosine (purine), thiamine, threonine, and tyrosine, respectively; \textit{lac}, \textit{gal}, \textit{mal}, \textit{xyl}, and \textit{mtl} denote inability to ferment lactose, galactose, maltose, xylose, and mannitol, respectively; \textit{Sm} SmR and \textit{Sm} T6X denote resistance to streptomycin and phage T6, respectively; all strains not marked \textit{Sm} denote streptomycin-sensitive; (\textlambda{}) denotes strains lysogenic for \textlambda{}; RNAse I+ indicates strains which lack ribonuclease I.}
\end{table}

\begin{figure}
\centering
\includegraphics[width=\textwidth]{fig1.png}
\caption{Genetic map of the \textit{E. coli} chromosome [from Taylor and Trotter (18)]; \textit{thr} and \textit{argG} are transferred early by \textit{Hfr}H and \textit{AB312}, respectively.}
\end{figure}
TABLE 2. Three-point mapping of asp, argG, and pnp by P1(Q13) transduction of AA1 (Asp+ Arg+)

<table>
<thead>
<tr>
<th>Selected marker</th>
<th>Scored markers</th>
<th>Percentage of scored markers</th>
</tr>
</thead>
<tbody>
<tr>
<td>Asp+</td>
<td>Arg+ pnp-13</td>
<td>30</td>
</tr>
<tr>
<td>Asp+</td>
<td>Arg+ pnp-13</td>
<td>11</td>
</tr>
<tr>
<td>Asp+</td>
<td>Arg- pnp-13</td>
<td>2</td>
</tr>
<tr>
<td>Asp+</td>
<td>Arg- pnp+</td>
<td>57</td>
</tr>
<tr>
<td>Arg+</td>
<td>pnp-13</td>
<td>77</td>
</tr>
<tr>
<td>Arg+</td>
<td>pnp+</td>
<td>23</td>
</tr>
</tbody>
</table>

Fig. 2. Cotransduction frequencies of asp, argG, and pnp.

with T6, and scoring for Sm (Fig. 3). Of 240 recombinants scored, 71% were Sm+, indicating that asp rather than argG was nearer to str and thus supporting the order str asp argG pnp. This is in agreement with the orientation of asp argG determined by R. Lavelle (personal communication).

Explanation for inconsistencies observed in transduction of asp. The high linkage (41%) observed between asp and argG in transduction of AA1 (asp argG, etc.) to Asp+ Arg+ is not demonstrated in all transductions. For example, P1 grown on PA3403, a strain with the identical asp lesion as AA1, does not donate asp (<0.2%) in the transduction of AB1302 (argG, etc.) or of AT12-55 (argG, etc.) to Arg+. In transducing PA3403 (asp, etc.) to Asp+, P1(AT12-55) donates argG with 30% frequency, whereas P1(AB1302) does not donate argG (<0.2%). (Conjugation experiments confirm that the Arg- defects of AT12-55 and AB1302 map at argG.)

These observations can be explained if AB1302 and AT12-55 contain a gene unlinked to argG which suppresses the donated asp mutation. To see whether such a gene existed, I looked for an Hfr strain which could convert AA1 (asp argG, etc.) to Asp+ by transferring a region of the chromosome unlinked to argG. HfrH strain AT2446 was found to do so. In an interrupted mating of AT2446 × AA1, the Asp+ phenotype is transferred about 20 min after lac, unlinked to argG+ transfer. In addition, P1(AT2446) cotransduces argG and asp.

Table 3 shows the aspartate genotypes of relevant strains if an asp and an asp-suppressing locus are assumed. For Asp- strains, both alleles are negative. For Asp+ strains, the asp genotype linked to argG is determined by whether the strain transfers its argG allele in transduction of AA1 or PA3403 to Asp+. The asp-suppressing genotype is determined for AB1302 and AT12-55 by their inability to express asp from PA3403 in Arg+-selected transduction, and for HfrH strains AT2446 and A19 by their respective ability and inability to transfer in conjugation with AA1 an Asp+ function unlinked to argG. This work does not distinguish whether this gene is involved in aspartate metabolism or is a nonsense or missense suppressor. Since neither A19 (the parental strain of Q7, Q13, and Q27) nor AA1 suppresses asp, the cotransduction frequencies obtained for asp, argG, and pnp are unaffected.

DISCUSSION

To isolate PNPase mutants without knowing a physiological function for the enzyme, an assay was devised which could measure the PNPase level of 100 clones/day, by using patches of cells grown overnight. Since heavily mutagenized strains were used, the numbers of cells in each patch which grew up overnight fluctuated. To eliminate low activity levels due to reduced cell quantity the assays were run considerably longer than the time normally required for the ADP-3P exchange reaction to approach equilibrium. This might have masked certain mutants with reduced PNPase activity by allowing them a longer effective reaction time. Nevertheless, three mutants were isolated from 950 strains tested.

If PNPase were an essential enzyme, cells devoid of it could be isolated only as conditional lethals. To detect mutants with temperature-sensitive PNPase, suspended cells were preincubated at 43°C for 1 hr during toluene treatment.

Fig. 3. Three-point cross ordering str asp argG. (Sm is the phenotypic designation for the str gene.)

TABLE 3. Inferred aspartate genotypes for the str gene.

<table>
<thead>
<tr>
<th>Strain</th>
<th>asp locus linked to argG</th>
<th>asp-suppressing locus linked to argG</th>
<th>Aspartate phenotype</th>
</tr>
</thead>
<tbody>
<tr>
<td>AB1302</td>
<td>-</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>AT12-55</td>
<td>+</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>PA3403</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>AA1</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>A19</td>
<td>+</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>AB2446</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
</tbody>
</table>
and then were assayed at 43 C. In addition, during part of the mutant search the screening pool was enriched with temperature-sensitive strains. In this way, Q27 was isolated.

Nitrosoguanidine has recently been reported to mutagenize preferentially the growing zone of the E. coli chromosome (2). In nitrosoguanidine mutagenesis of stationary-phase cells, Botstein (Cold Spring Harbor Symp. Quant. Biol., in press) found that mutations cluster exclusively in one-third of the chromosome near the region where replication starts. The observation that pnp maps in the middle of that region is suggested as an explanation for the fortuitously high frequency of PNPase mutations which I obtained.

The Q13 coenzyme A artifact indicates the difficulty of attempting to study a mutation in a heavily mutagenized strain containing other known and unknown lesions. Characterization of the PNPase mutations in nearly isogenic strains is presented in the accompanying paper (13).

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LITERATURE CITED