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Polynucleotide Phosphorylase Plays an Important Role in the Generation of Spontaneous Mutations in *Escherichia coli*

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Polynucleotide phosphorylase (PNP) plays a central role in RNA degradation, generating a pool of ribonucleoside diphosphates (rNDPs) that can be converted to deoxyribonucleoside diphosphates (dNDPs) by ribonucleotide reductase. We report here that spontaneous mutations resulting from replication errors, which are normally repaired by the mismatch repair (MMR) system, are sharply reduced in a PNP-deficient *Escherichia coli* strain. This is true for base substitution mutations that occur in the *rpoB* gene leading to Rif^r and the *gyrB* gene leading to Nal^r and for base substitution and frameshift mutations that occur in the *lacZ* gene. These results suggest that the increase in the rNDP pools generated by polynucleotide phosphorylase (PNP) degradation of RNA is responsible for the spontaneous mutations observed in an MMR-deficient background. The PNP-derived pool also appears responsible for the observed mutations in the *mutT* mutator background and those that occur after treatment with 5-bromodeoxyuridine, as these mutations are also drastically reduced in a PNP-deficient strain. However, mutation frequencies are not reduced in a *mutY* mutator background or after treatment with 2-aminopurine. These results highlight the central role in mutagenesis played by the rNDP pools (and the subsequent dNTP pools) derived from RNA degradation.

Elucidating the pathways that lead to mutations resulting from replication errors, arising either spontaneously or induced by chemical agents, has intrigued molecular biologists ever since the elucidation of the structure of DNA allowed one to pose this question in molecular terms (57, 58). What is the actual source of these mutations? Different tautomeric forms of the four bases in DNA or their analogs have been considered which provoke errors directly (18, 24, 28, 29, 57) or even indirectly, for instance by affecting the pools of nucleoside triphosphates (28, 29, 34). Also, the field has defined a myriad of repair systems aimed at preventing or repairing DNA damage (24) and also aimed at correcting errors of DNA replication (30, 47). In humans, defects in one of a number of repair systems leads to inherited cancer susceptibilities (e.g., see references 1 and 22). In *Escherichia coli*, the replicating DNA polymerase (Pol III) contains an editing subunit that corrects numerous replication errors (14, 19, 41). Directly after replication, the mismatch repair (MMR) system recognizes still-uncorrected mismatches and repairs them using the pattern of methylation to distinguish the template strand from the newly synthesized strand (30, 47). Mutants lacking any one of the components of this system (e.g., MutH, MutL, MutS, UvrD) have sharply elevated mutation rates that involve transitions (A:T→G:C or G:C→A:T) (11, 36, 54) or short indels (insertion/deletions) at repeated sequences, such as monotonous runs of G's or A's on the same strand (10, 54). The size and balance of the nucleoside triphosphate (NTP) pools are important for replication fidelity (33). Not only do unbalanced pools provoke an increase in mutagenesis (33, 38, 44), but an increase in the pools of all four dNTPs also leads to increased mutations (26, 59).

One widely used approach to studying mutational pathways is to find mutants with increased mutation rates, or "mutators" (e.g., see references 43 and 46). A more difficult approach is to detect mutants with lowered mutation rates, or "antimutators." In a study to be reported elsewhere (E. Becket and J. H. Miller, unpublished), we screened the *E. coli* Keio collection of gene knockouts for mutants with a reduced rate of mutagenesis induced by the base analog 5-azacytidine (5AZ). We found that mutants deleted for the *pnp* gene, encoding polynucleotide phosphorylase (PNP), had lower levels of 5AZ-induced mutagenesis. Here, we report the effects of PNP deficiency on mutagenesis induced by two other base analogs, 5-bromodeoxyuridine (5BdU) and 2-aminopurine (2AP), as well as on spontaneous mutations that result from replication errors. These studies indicate that the pool of nucleoside diphosphates generated by PNP-mediated degradation of RNA drives spontaneous and certain mutagen-induced mutations.

MATERIALS AND METHODS

***E. coli* strains.** The Keio collection is as described by Baba et al. (3) and made from the starting strain BW25113 (13). This strain (*lacI*^{q} *rrnB*$_{T14}$ Δ*lacZ*$_{WJ16}$ *hsdR514* Δ*araBAD*$_{AH33}$ Δ*rhaBAD*$_{LD78}$) is the starting strain used in the experiments reported here unless otherwise stated. Briefly, each of the 3,985 strains in the Keio collection carries a complete deletion of a different gene, with a *kan* insert replacing each gene. The base substitution tester strains CC102 and CC106 have been described previously (11). They carry a base substitution mutation in *lacZ* on the F′ plasmid, which reverts from Lac$^-$ to Lac$^+$ only by restoring the glutamic acid codon through a reversion by a specific base substitution for each (G→A, G→C, and A→G, respectively). Additionally, these strains carry a miniTn*10-cat* insert (45) conferring chloramphenicol resistance for selection purposes (C. Tamae and J. H. Miller, unpublished). Also, *mutL*, *mutT*, and *mutY* derivatives of BW25113 were prepared by P1 transduc-

Received 31 May 2012 Accepted 6 August 2012
Published ahead of print 17 August 2012
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doi:10.1128/JB.00962-12

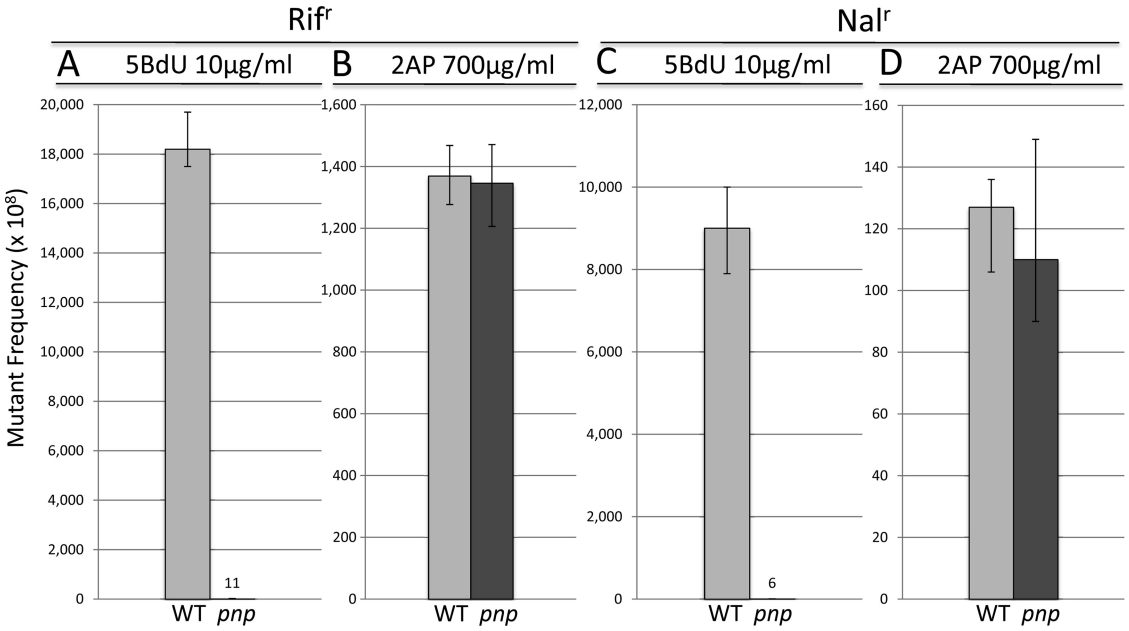

FIG 1 Mutagenesis in WT and PNP-deficient strains. Mutant frequencies for 5BdU- and 2AP-induced mutations in *rpoB*, generating Rif^r mutants, and *gyrA*, generating Nal^r strains, are shown. Error bars reflect 95% confidence limits (see Materials and Methods for further details).

tion from strains carrying miniTn*10-tet* inserts (Tamae and Miller, unpublished) in either *mutL*, *mutT*, or *mutY*.

***E. coli* genetic methods.** Unless otherwise stated, all genetic methods are as described by Miller (45).

Validation controls. A collection of close to 4,000 strains will contain some errors and some impure strains. The latter problem can be minimized by repurifying and retesting, as was done here. Yamamoto and coworkers have subjected the Keio collection to an intensive analysis aimed at uncovering errors in the collection that might arise from duplications of the target gene. They generated a list of 14 mutants that are incorrect and another 9 that might be incorrect (61). Ultimately, the most prudent use of such a large collection is to verify any mutants that are particularly important to the final results by PCR analysis, and/or sequencing, as we have done in a number of cases (4, 37). Here, using PCR and sequencing we have verified the strain with a deletion of the *pnp* gene. Using a primer within the *kan* gene and a primer outside the gene it replaced, we showed that a *kan* insert was at the correct position. Using internal primers for the *pnp* gene, we showed that there was no other copy of the *pnp* gene elsewhere in the chromosome. Controls with the starting wild-type (WT) strain showed that the internal primers were efficient.

We transduced the *pnp* deletion/*kan* insertion-carrying strain used in these experiments with a linked Tn*10* element (from strain CAG12153 [55]). A majority of the Tet^r transductants had crossed out the *pnp* deletion/replacement, as determined by the loss of Kan^r, which was accompanied by the restoration of the normal colony size. We tested two of these transductants for the mutation frequency (Rif^r) in the presence of 5BdU and found that they had restored the high mutation frequency characteristic of PNP-proficient strains (data not shown).

The major hot spot in *rpoB* that occurs after 5BdU treatment or in MMR-deficient strains represents two-thirds of the mutations detected (see Fig. 3 and reference 25). To verify that cells with this mutation still confer the Rif^r phenotype in a PNP-deficient strain, we carried out a reconstruction experiment. We transduced this mutation from the wild type into a *pnp* derivative in two steps without selecting for Rif^r by using a linked Tn*10* in the *argE* gene (from strain CAG12185 [55]). First, *argE*::Tn*10* was transduced into the *pnp* deletion/*kan* replacement strain, scoring for retention of the Kan^r phenotype. Then with a lysate prepared on the Rif^r strain carrying the *rpoB* allele in question, the strain was transduced to Arg$^+$. Arg$^+$ transductants were scored for retention of the *pnp*/*kan* deletion/replacement and also for Rif^r. The expected 60% of the Arg$^+$ strains formed Rif^r colonies (in the *pnp* background).

Stock solution and cell treatment. 5-Bromodeoxyuridine was prepared by dissolving in distilled water to a concentration of 2 mg/ml. Solid 2-aminopurine was added directly to LB medium for a concentration of 700 μg/ml, which was then distributed in 3-ml aliquots and followed by the addition of ~5×10^4 cells and incubation for 24 h at 37°C with aeration.

Determination of mutation frequencies. We inoculated 100 to 1,000 cells in a series of cultures of LB or LB plus 2-aminopurine (2AP) or 5BdU, where they were grown for 18 h at 37°C with aeration prior to plating on the appropriate medium (lactose-minimal plates, or LB plates with either 100 μg/ml rifampin or 20 μg/ml nalidixic acid). The mutation frequencies of Rif^r and Lac$^+$ revertants were determined as described previously (44). Briefly, the mutation frequency (f) was determined as the median frequency from a set of cultures (the number of cultures varied from 8 to 20), and the mutation rate (μ) was determined by the formula of Drake (17). We determined the 95% confidence limits according to the method of Dixon and Massey (15).

Chemicals. 5-Bromodeoxyuridine, 2-aminopurine, tetracycline, chloramphenicol, and kanamycin were purchased from Sigma (St. Louis, MO).

RESULTS

Base analog-induced mutagenesis. Fig. 1A shows that 5BdU mutagenesis is reduced in a *pnp* mutant on the order of 1,000-fold relative to the PNP$^+$ starting strain, as measured by the frequency of Rif^r mutants. However, 2AP mutagenesis is not lowered in a *pnp* mutant (Fig. 1B). We have verified the effect of a PNP deficiency on mutagenesis in two additional systems. Figure 1C shows that when mutagenesis was monitored by the frequency of nalidixic acid resistance (Nal^r), 5BdU again displayed a reduction on the order of 1,000-fold in a *pnp* strain, whereas 2AP mutagenesis was not reduced (Fig. 1D). More-

TABLE 1 Frequencies (*f*) and mutation rates (μ) in CC102 and CC106 after treatment with 5BdU at 10 μg/ml[a]

Strain	Frequency (*f*) ($\times 10^8$)	Mutation rate (μ) ($\times 10^8$)
CC102 WT	470 (420–1,300)[b]	60 (54–143)
CC102 *pnp*	3 (0–7.5)	0.86 (0–1.8)
CC106 WT	760 (410–1,900)	90 (53–206)
CC106 *pnp*	0 (0–1.7)	0 (0–0.57)

[a] The *rpoB* mutation frequencies (*f*) per cell were calculated by dividing the median number of mutants by the average number of cells in a series of cultures, and the mutation rates (μ) were determined with the method of Drake (17) (see Materials and Methods).
[b] Values in parentheses are 95% confidence limits.

over, use of the reversion of specific *lacZ* alleles to measure mutagenesis revealed that 5BdU-induced mutagenesis was also sharply reduced (Table 1). CC102 reverts to wild type via a specific G:C→A:T transition, and CC106 reverts via a specific A:T→G:C transition (11). Because PNP and RNase II (RNB) are the two enzymes responsible for RNA degradation and the resulting ribonucleoside diphosphate (rNDP) and rNMP pools (12, 16), respectively, derived from this degradation, we tested *rnb* (encoding RNB) mutants. Figure 2 shows that a deletion of *rnb* also resulted in a reduction of 5BdU-induced mutagenesis, although the modest 3.5- to 4.0-fold decrease was far less than that seen with *pnp* deletion strains.

Pathways of 5BdU-induced mutagenesis. The study of mutagenic spectra has revealed that spontaneous mutations, as well as those induced by each mutagen treatment and mutator background, result in a characteristic pattern of hot spots and cold spots (e.g., see references 5, 8, 21, and 25), most probably as a result of the surrounding sequences. Analysis of the spectra of mutations in *rpoB* leading to Rif^r is a case in point (25, 35). Figure 3 shows the pattern of transition mutations (A:T→G:C, G:C→A:T) in *rpoB* for the base analogs 2AP (25), zebularine (ZEB) (35), and 5BdU (this work), as well as the mutagen UV irradiation (UV) (25), the mismatch-repair-deficient mutator *mutS* (25), and the unmutagenized wild type (25, 60). There are 29 sites that have been detected in *rpoB* that lead to Rif^r via a transition (60), and these are listed by base pair number in *rpoB* in terms of the percentage of the total number of Rif^r mutants analyzed. The A:T→G:C mutations are on the left portion of each panel, and the G:C→A:T mutations are on the right portion of each panel. The total numbers of mutations analyzed in each sample were 30 (2AP), 156 (ZEB), 194 (5BdU), 40 (UV), 174 (*mutS* [MMR$^-$]), and 298 (SPON). The percentage of the total mutations that resulted from transitions was close to or more than 90% for all but the SPON (WT) set. These percentages were 97% (2AP), 92% (ZEB), 99% (5BdU), 88% (UV), 98% (*mutS*), and 47% (SPON). What is evident from Fig. 3 is that while each mutagen or mutator shows a different pattern of transition frequencies at the available sites, 5BdU displays a pattern that is virtually identical to that of a mismatch-repair-deficient strain (*mutS*). Although one might argue that this pattern is coincidental, we see the same pattern among sites in the *gyrB* gene that lead to Nal^r. Table 2 displays our analysis of *gyrB* mutations leading to Nal^r. There are fewer sites in *gyrB* than in *rpoB*, but of the 18 sites detected, 8 involve transitions, and 4 of these represent the A:T→G:C transition that is favored by 5BdU in the *rpoB* gene (Fig. 3). One of these sites, at position 247, is the prominent hot spot in MMR-deficient strains (*mutL*) and after 5BdU treatment. Note that 2AP does not have the same hot spot. Moreover, overproducing MutL on a plasmid reduced the level of 5BdU mutagenesis up to 150-fold (data not shown). The results with 5AZ, and for *mutT* and *mutY*, are also shown here to underscore the fact that this system correctly identifies the known mutagenic specificity of these treatments or strain backgrounds. Namely, 5AZ is specific for G:C→C:G changes, *mutT* for A:T→C:G changes, and *mutY* for G:C→T:A changes (9, 11, 35, 48). Taken together, the results shown in Fig. 3 and Table 2 strongly indicate that 5BdU is not targeting mutations directly, as do other base analogs, such as 2AP, ZEB, and 5-azacytidine, but instead acts indirectly by increasing normal DNA polymerase replication errors and saturating mismatch repair.

Spontaneous base substitution mutations. We examined the effects of deleting *pnp* on spontaneous mutations. While the effects on the frequency of spontaneous base substitution mutations in an MMR-proficient background are minimal (Table 3), the effects in an MMR-deficient strain are dramatic, as foreshadowed by the results with 5BdU. Figure 4 shows that in three different systems the introduction of a *pnp* deletion results in a decrease of Rif^r mutations by 140-fold, Nal^r mutations by 160-fold, and Lac$^+$ mutations resulting from an A:T→G:C transition in *lacZ* by 64-fold and from a G:C→A:T transition by 78-fold. These levels are very near to those seen in an MMR-proficient strain (Table 3). To demonstrate that these reductions are not simply an artifact of the *pnp* background, we showed that the increase in mutation frequency that occurs in a *mutY* background (48) is not reduced in a *mutY pnp* background (Fig. 5A and B). However, the increased level of mutations in a *mutT* mutator background is eliminated when *pnp* is deleted (Fig. 5C and D), as the levels are now close to those seen in a wild-type strain.

Frameshift mutations. Frameshift mutations at repeated

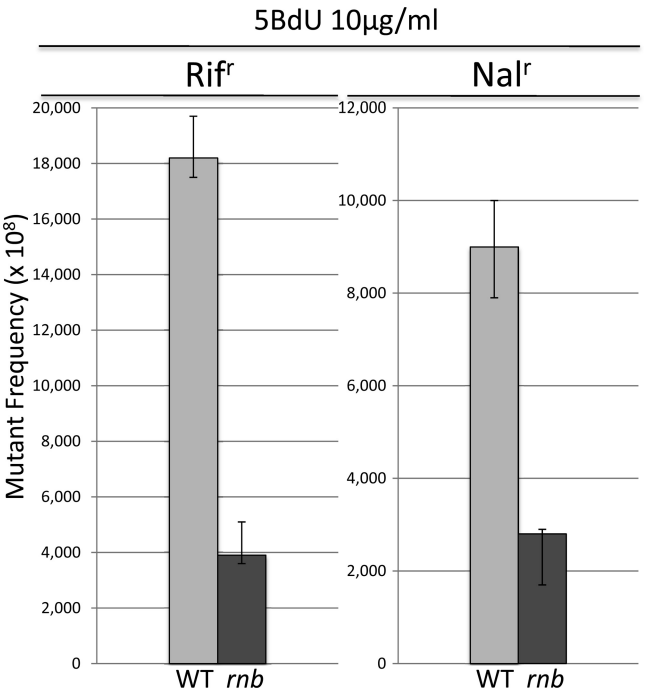

FIG 2 5BdU mutagenesis in WT and RNB-deficient strains (see legend to Fig. 1).

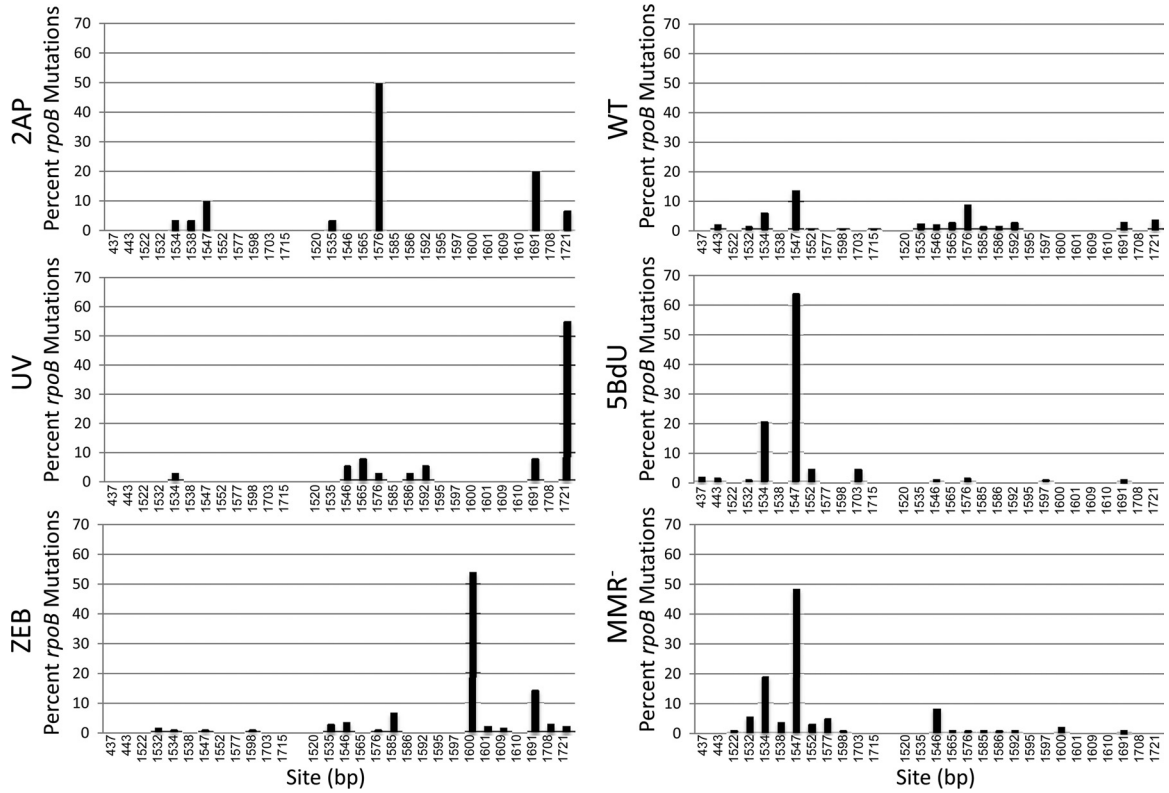

FIG 3 Transition mutations in *rpoB* leading to the Rif^r phenotype occurring spontaneously or after treatment with different mutagens. A:T→G:C mutations are shown in the left portion of each diagram, and G:C→A:T mutations are shown in the right portion. The height of each bar represents the percentage of all the *rpoB* mutations detected in that sample. For further details, see Results.

mono- or dinucleotides are frequent replication errors that are repaired by the MMR system (10, 30, 54). Strain CC107 reverts to Lac$^+$ via the addition of a G in a monotonous run of 6 G's (10). Table 4 shows the frequency of this frameshift mutation in cultures of wild-type and MMR-deficient strains. 5BdU treatment also increases the level of these frameshifts to near the level of that seen in an MMR-deficient strain. Strains deleted for *pnp* reduce the levels of this mutation 330-fold in MMR-deficient strains and in 5BdU-treated cells down to even below the level of untreated wild-type strains, a several-thousand-fold effect (Table 4).

TABLE 2 Distribution of mutations in *gyrA*

Site (bp)	AA change	bp change	WT	WT 5BdU	WT 2AP	*mutL*	*mutT*	*mutY*	WT 5AZ
215	D→G	AT→GC	0	2	0	0	0	0	0
245	D→G	AT→GC	1	1	0	5	0	0	0
247	S→P	AT→GC	0	0	0	1	0	0	0
260	D→G	AT→GC	17	57	2	67	0	0	2
152	A→V	GC→AT	0	0	0	0	0	0	0
244	D→N	GC→AT	0	0	2	0	0	0	0
248	S→L	GC→AT	17	0	8	10	0	1	0
259	D→N	GC→AT	5	0	8	1	0	0	0
241	G→C	GC→TA	2	0	0	0	0	1	0
259	D→Y	GC→TA	11	0	0	0	0	0	0
356	A→E	GC→TA	0	0	0	0	0	6	0
245	D→A	AT→CG	0	0	0	0	0	0	0
247	S→A	AT→CG	1	0	0	0	7	0	0
260	D→A	AT→CG	1	0	0	0	19	0	0
260	D→V	AT→TA	0	0	0	0	0	0	0
248	S→W	CG→GC	2	0	0	0	0	0	31
259	D→H	CG→GC	1	0	0	0	0	0	4
316	Q→E	CG→GC	0	0	0	0	0	0	0
Total			55	60	20	84	26	8	37

DISCUSSION

We show here that deleting the gene (*pnp*) encoding polynucleotide phosphorylase (PNP) virtually eliminates the dramatic increase of spontaneous mutations that occurs when the mismatch repair (MMR) system is inactivated (e.g., in *mutS* or *mutL* strains). This is true for base substitution mutations (Fig. 4) that occur in the *rpoB* gene leading to Rif^r or in the *gyrB* gene leading to Nal^r and for both base substitutions and frameshift mutations that occur in the *lacZ* gene (Tables 1 and 4). This is also true for base substitutions resulting from inactivation of the *mutT* gene (Fig. 5), which sanitizes the pool of oxidized dGTPs (39) and rGTPs (56) by converting them to monophosphates. Mutations that are elevated in a MutY-deficient background, however, are not decreased (Fig. 5), as the mutations result from oxidation of guanines in the DNA itself. Moreover, there is a similar striking reduction in mutations generated after continuous growth in 5BdU (Fig. 1; Table 4). Although 5BdU is incorporated into DNA, it has been suggested that it may cause mutations indirectly, rather than directly targeting the mutations (28, 29, 34). An inspection of the mutational

TABLE 3 Spontaneous frequencies (f) and mutation rates (μ) in wild-type and PNP-deficient strains[a]

	Rif^r		Nal^r	
	WT	PNP-deficient strain	WT	PNP-deficient strain
f ($\times 10^8$)	3.7 (3.0–4.6)[b]	2.9 (2.3–3.8)	0.46 (0.23–0.69)	0.39 (0–0.79)
μ ($\times 10^8$)	0.81 (0.68–0.96)	0.68 (0.57–0.85)	0.15 (0.090–0.21)	0.14 (0–0.24)

[a] The *rpoB* mutation frequencies (f) per cell were calculated by dividing the median number of mutants by the average number of cells in a series of cultures, and the mutation rates (μ) were determined with the method of Drake (17) (see Materials and Methods).
[b] Values in parentheses are 95% confidence limits.

spectra in *rpoB* argues that 5BdU induces mutations indirectly by enhancing normal replication errors and saturating mismatch repair (Fig. 3; see text). In contrast, 2AP-induced mutations are not decreased in a PNP-deficient strain (Fig. 1).

How can the absence of PNP virtually eliminate spontaneous mutagenesis under certain conditions? PNP plays a central role in RNA degradation, generating a pool of rNDPs (12, 16) that mix with the pools from *de novo* biosynthesis (Fig. 6) (42, 52). One should note that *de novo* pyrimidine biosynthesis is carried out at a low level, the rate-limiting step being the conversion of UMP/CMP to UDP/CDP, respectively (12). The primary source of pyrimidines for DNA synthesis may in fact be the pool of nucleosides derived from RNA (12). There is 10-fold more RNA in the in the cell than DNA (20), and the ribonucleoside pools have been measured to greatly exceed deoxyribonucleoside pools. For instance, in yeast, the four rNTPs are 36 to 190 times more prevalent than the four dNTPs (49). Also, because ribonucleosides are converted to deoxyribonucleosides by ribonucleoside diphosphate reductase (RNR) at the diphosphate level, the rNDPs generated by PNP-mediated degradation of RNA can be easily shunted into DNA (Fig. 6). We therefore can envision several explanations for the effect on mutagenesis of deleting *pnp*. One possibility is that the PNP-generated pools normally result in the incorporation of ribonucleosides into DNA and that this has mutagenic consequences. Direct measurement of ribonucleosides incorporated into DNA by yeast DNA polymerases *in vitro* indicates that rNMP is incorporated from once for every 625 dNMPs to once per 5,000 dNMPs, depending on the polymerase (49). These are removed *in vivo* by a process involving RNase H2, and mutants lacking RNase H2 in yeast have increased levels of spontaneous mutations that involve primarily short deletions or insertions of 2 to 5 bp (6, 7, 53). Models for the generation of these mutations involve processing by topoisomerase I, and misalignment (32). Interestingly, these mutations are not increased in an MMR-deficient strain (7). However, this mechanism would not account for most of the mutations that we score in the work reported here, as these are base substitution mutations that are subject to mismatch repair. Moreover, in the *E. coli* strain background we are using, RNase H1- or RNase H2-deficient strains (*rnhA* or *rnhB*) are not mutators for the

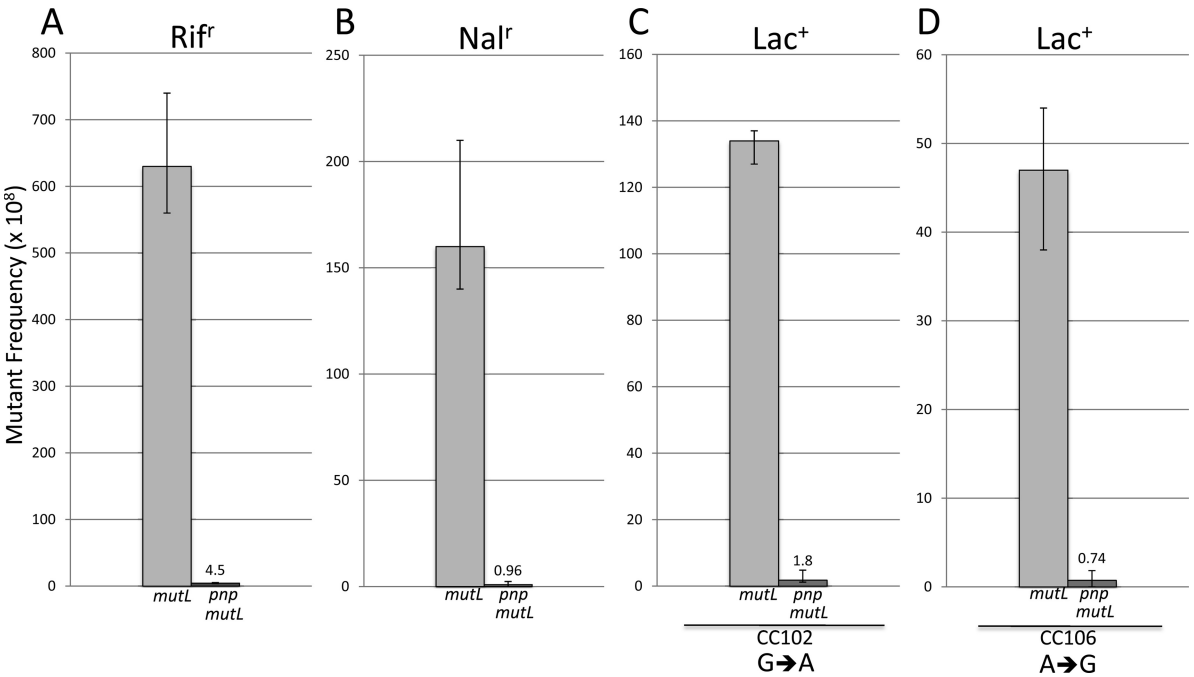

FIG 4 Mutant frequencies of MutL-deficient strains in otherwise WT or PNP-deficient backgrounds. The frequencies of Rif^r, Nal^r, or Lac$^+$ mutants are shown in each case, reflecting mutations in *rpoB*, *gyrA*, and *lacZ*, respectively (see legend to Fig. 1 and text).

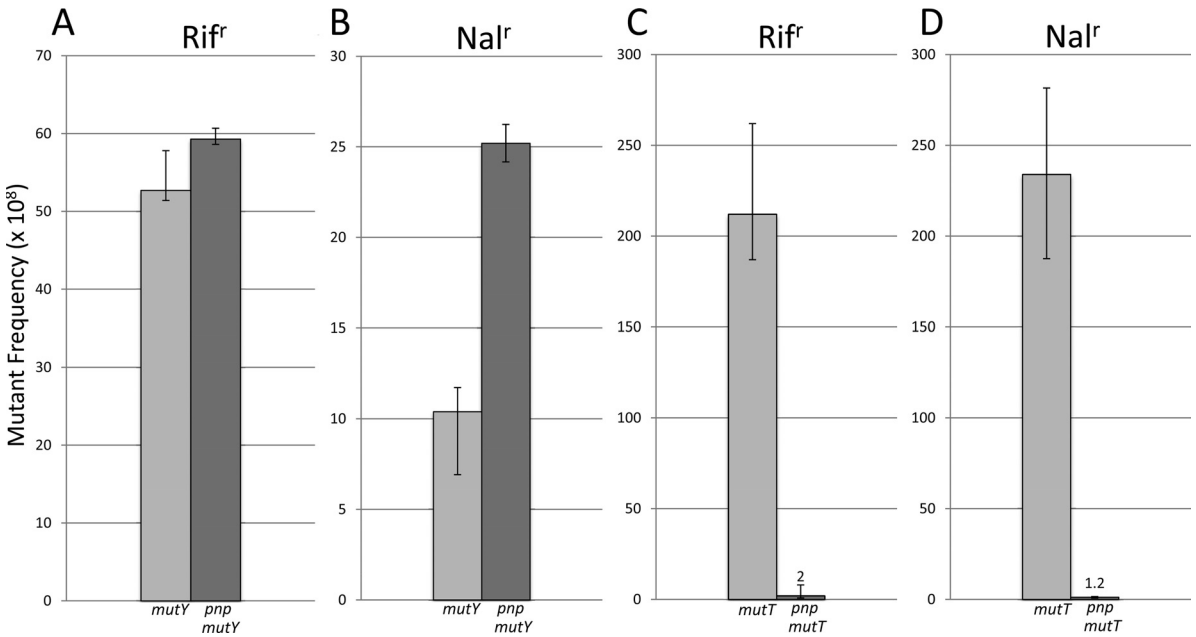

FIG 5 Mutant frequencies in WT and PNP strain backgrounds that are also either MutY or MutT deficient (see legend to Fig. 1 and text).

rpoB system used here in either an otherwise wild-type background or an MMR-deficient background (data not shown).

A second and related possibility is that degradation of RNA by PNP provides uracil in the form of rUDP. This is a required intermediate in the incorporation of dTTP into DNA. The rUDP is converted to dUDP and then dUTP, and then dUTPase converts dUTP to dUMP, from which it is converted into dTMP. dUTPase action prevents dUTP from being incorporated into DNA. Mutants with reduced activity of dUTPase have significant uracil incorporated into DNA, but this is normally not mutagenic (e.g., see reference 51). However, under certain circumstances the additional uracil can be mutagenic, such as when a reduced-activity dUTPase mutant also carries a mutation in the *ndk* gene (51). Nordman and Wright have argued that NDK is involved in removing uracil from DNA (51). It could simply be, however, that NDK-deficient cells are just beginning to saturate MMR, and the combination of NDK deficiency and reduced dUTPase activity titrate out MMR, with the result being the observed higher error rate. It is possible that, in this scenario, under normal conditions in a wild-type strain a certain amount of uracil could get into the DNA and cause replication errors that are few enough to be corrected by mismatch repair, but are revealed in an MMR-deficient strain. Then, in a PNP-deficient strain, the reduced pools of rUDP would result in greatly reduced polymerase errors, and a reduced mutation frequency. Arguing against this, however, is our demonstration that for at least the main hot spot in *rpoB*, the key intermediate is an A:C mispair, and not a G:T or G:U mispair (31). Also, the finding that *mutT*-stimulated mutations are greatly reduced in a *pnp* background is not explained by increases in uracil in the DNA (Fig. 5).

TABLE 4 Frequencies (*f*) and mutation rates (μ)[a] of frameshift mutations with and without treatment with 5BdU at 10 μg/ml

Strain	Frequency (*f*) (×10^8)	Mutation rate (μ) (×10^8)
Without 5BdU		
CC107 WT	56 (45–79)	8.9 (7.4–12)
CC107 *mutL* mutant	76,000 (69,000–78,000)	5,900 (5,300–6,000)
CC107 *pnp mutL* mutant	230 (180–360)	30 (24–45)
With 5BdU at 10 μg/ml		
CC107 WT	27,000 (23,000–34,000)	2,200 (1,900–2,700)
CC107 *pnp* mutant	7 (4.5–8.8)	1.5 (1.0–1.8)

[a] The *rpoB* mutation frequencies (*f*) per cell are calculated by dividing the median number of mutants by the average number of cells in a series of cultures, and the mutation rate (μ) was determined by the method of Drake (17) (see Materials and Methods). Values in parentheses are 95% confidence limits.

Perhaps the most likely model involves the concept that the levels of the pools of all four canonical bases in DNA determines the rate of DNA replication (27) and mutagenesis. Several groups have shown that plasmid overexpression of RNR results in increased levels of dNTPs and increased mutagenesis (26, 59). Moreover, a deletion of the *cmk* gene in *E. coli* results in reductions in the dCTP pools (to 30% of the wild-type level) and the dTTP pools (to 70% of the wild-type level), and a subsequent 2-fold reduction of the replication elongation rate (23). A number of studies have also shown that in higher cells the nucleotide pools affect the speed of replication. In mammalian somatic cells a reduction in nucleotide availability results in a lowering of the rate of fork progression (2). In mouse embryos, the rates of DNA synthesis are closely correlated with the intracellular deoxynucleotide pools during embryonic development (50). In synchronized populations of human HeLa cells, the addition of exogenous dNTPs accelerated the speed of replication fork movement in early S phase (40). Given the link that these studies show between nucleotide pools and both replication speed and mutagenesis, our results here suggest that in a wild-type strain, the dNDP pools em-

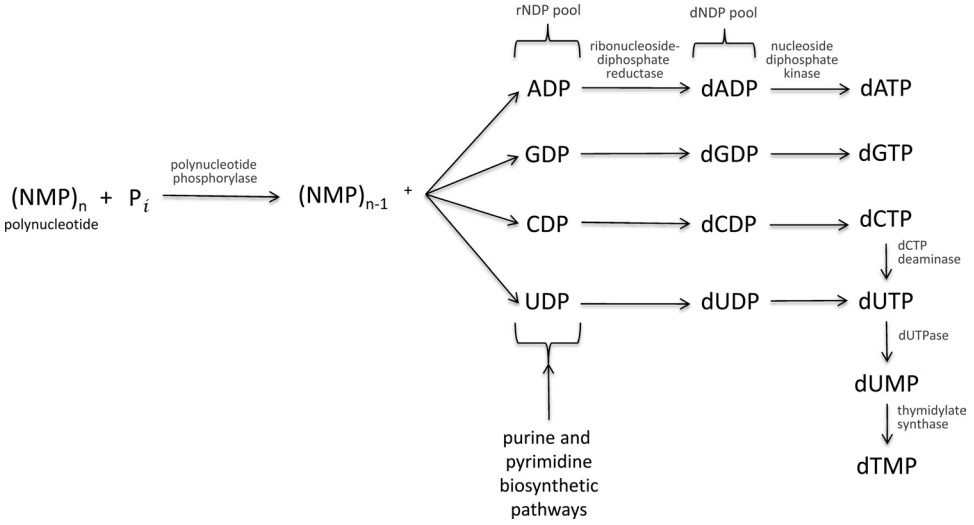

FIG 6 Pathways emanating from RNA biosynthesis and degradation (42, 52).

anating from rNDPs generated by PNP (Fig. 6) generate replication errors that are repaired by the MMR repair system. Elimination of the additional rNDP pool created by PNP, by deleting the *pnp* gene, sharply reduces these errors so that even in an MMR-deficient strain, one does not observe mutations much over the background.

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