Antimutator Mutants in Bacteriophage T4 and *Escherichia coli*

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ABSTRACT

Antimutators are mutant strains that have reduced mutation rates compared to the corresponding wild-type strain. Their existence, along with mutator mutants that have higher mutation rates compared to the wild-type strain, are powerful evidence that mutation rates are genetically controlled. Compared to mutator mutants, antimutators have a very distinguishing property. Because they prevent normally occurring mutations, they, uniquely, are capable of providing insight into the mechanisms of spontaneous mutations. In this review, antimutator mutants are discussed in bacteriophage T4 and the bacterium *Escherichia coli*, with regard to their properties, possible mechanisms, and implications for the sources of spontaneous mutations in these two organisms.

ANTIMUTATORS are genetic mutants that produce mutations at reduced rates compared to the wild-type strain. They are important because they, uniquely, can provide insights into the mechanisms by which spontaneous mutations occur. Mechanistically, antimutators can be thought to increase the efficiency of the normal mutation-prevention systems or, alternatively, decrease the efficiency of error-producing systems. In either case, an understanding of the mechanism by which the antimutator reduces spontaneous mutations provides a direct insight into the mechanisms by which mutations occur in normal cells.

Historically, antimutator strains have received generally less attention than their counterpart, mutators, which produce mutations at elevated frequencies. One reason for this is that antimutators, because of their modest nature, are more difficult to detect and isolate than their highly visible mutator counterparts. In addition, it has been argued (Drake 1993) that the genetic potential for generating antimutators is lower than for generating mutators (see below). Both mutators and antimutators reveal aspects of the mechanisms by which organisms produce or prevent mutations, and both are relevant for understanding the underlying mechanisms. However, the two types of mutants present clearly different faces of this question. Mutators contain defects in pathways that cells use to prevent mutations, and their study therefore highlights *potential* sources of mutations (*i.e.*, mutations that could have occurred but do not). In contrast, antimutators can reveal the *actual* sources of spontaneous mutations, because their action prevents mutations that normally occur in wild-type cells when all mutation-prevention systems are operative.

In trying to understand spontaneous mutations, it is useful to realize that numerous potential sources for spontaneous mutations can be postulated. Figure 1 provides a schematic representation of some of the sources that have been considered. Within each pathway, several genes or gene products operate to reduce the contribution of this pathway. A defect in any of these will increase the mutations through this pathway, likely causing an overall mutator effect. This accounts for the observed multitude of mutator mutants. However, improving the efficiency of error prevention in any pathway, such as in a potential antimutator, while decreasing the number of mutations originating through this pathway, may not lead to a lowering of overall mutation. This will only occur if the particular pathway contributes substantially (*e.g.*, 50% or more) to overall mutations. Thus, the potential to generate antimutator mutants may be limited. Drake (1993) postulated that general antimutators (that lower overall mutation rates by a substantial fold) are improbable, based on both structural and evolutionary arguments. From an evolutionary perspective, selective pressures are not likely to lower mutations through one pathway much below the level of mutations afforded by other pathways; spontaneous mutations are therefore expected to be a mixture of quantitatively similar contributions. To what extent this hypothesis holds for organisms growing in a laboratory environment is an interesting question. The study of antimutator mutants may provide the best approach to answering this and related questions.

One technical aspect that complicates studies of antimutagenesis is that a perceived absence of mutations does not necessarily imply an antimutator effect. It is also possible that mutations are generated at normal frequencies but that they are somehow inefficiently expressed. This could be because of their incompatibility with the presumed antimutator allele or because of a general growth impairment under the selective conditions. Some possible examples of this will be indicated below.

In the following, I present an overview of some of the

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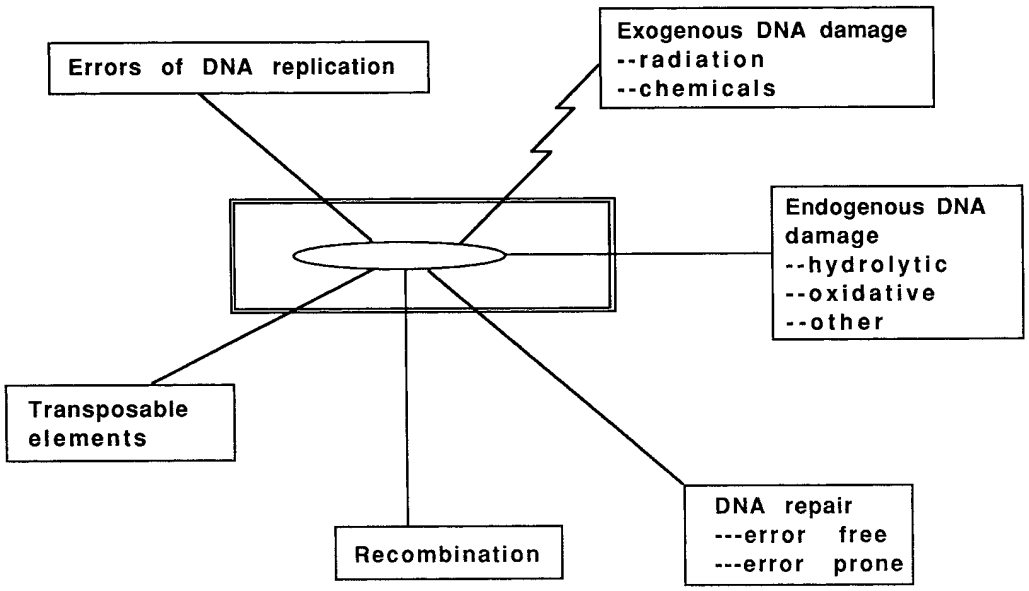

Figure 1.—Possible sources of spontaneous mutations. Each box represents an example of a potential mechanism by which mutations can arise. This representation is not exhaustive, and additional sources may be postulated. The pathways are not necessarily independent, as certain genes may function in more than one pathway. The main aim of this diagram is to stress that spontaneous mutations may represent a mixture of several contributing mechanisms and that pathway-specific antimutators may reveal the relative importance of these pathways.

properties of antimutators that have been isolated in bacteriophage T4 and in the bacterium *Escherichia coli*. Specifically, I will summarize what has been learned with regard to their possible mechanisms and what their implications are for the mechanisms of spontaneous mutations.

A. Antimutators in bacteriophage T4

In 1969, Drake and co-workers reported a survey of an extensive collection of temperature-sensitive (TS) mutants in gene *43* of bacteriophage T4, the gene encoding the T4 DNA polymerase (Drake *et al.* 1969). A large proportion of the TS mutants proved to be mutators, consistent with an earlier observation by Speyer (1965) who had demonstrated a mutator phenotype for the gp43 TS mutant *L56*. Interestingly, however, a number of the TS mutants appeared to be antimutator. The effects were only moderate in the T4*r* forward mutation assay but proved subsequently very strong in several *rII* reversion assays. This discovery of DNA polymerase mutants causing antimutator effects pointed very strongly to the importance of DNA polymerase fidelity in controlling spontaneous mutation, an important finding at that time. These findings sparked a decades-long interest in the mechanisms by which the antimutator effect could be achieved, as well as in the implications of the findings for the mechanisms by which the DNA polymerases achieve high fidelity of DNA replication.

The specificity of the T4 antimutators: One of the interesting properties of the T4 antimutators, already recognized in the 1969 *Nature* article, is that they have a defined specificity, *i.e.*, the effect is seen for certain mutations but not for others. It was observed that the antimutator effect was strong when measuring *rII* alleles reverting by A·T→G·C transition but not for those reverting by G·C→A·T transitions. Interestingly, when the G·C→A·T pathway was induced by the base analogue 5-bromouracil, a strong antimutator effect was readily observed.

Later studies on the specificity of the T4 antimutators were conducted by Ripley who showed that, similar to the G·C→A·T transitions, transversions were not reduced by the antimutator alleles. Instead, certain small increases in their frequencies were observed (Ripley 1975). Further studies also revealed an interesting dichotomy in the case of frameshift mutations (Ripley and Shoemaker 1983; Ripley *et al.* 1983). Frameshifts occurring in runs of identical bases were decreased in the antimutator mutants but frameshifts at many other sites (in general nonruns) were significantly enhanced. These specificity properties of the T4 antimutators are intriguing and need to be adequately explained by any proposed model describing the antimutator mechanisms (see below).

Possible mechanisms of the T4 antimutators: Muzyczka *et al.* (1972) accumulated early evidence that the antimutator mechanism did not simply reflect increased accuracy at the polymerization step but instead involved an altered interplay between the polymerase activity and the 3′-exonucleolytic proofreading activity. T4 DNA polymerase, like many other polymerases, contains as part of the same polypeptide a 3′→5′ exonuclease that functions as a proofreader for polymerase insertion errors. Muzyczka *et al.* (1972) showed that the purified *L141* and *L42* antimutator polymerases had a significantly greater ratio of exonuclease activity to polymerase activity (exo/pol ratio) than the wild-type enzyme which, in turn, had a significantly greater exo/pol ratio than two mutator enzymes tested (*L56* and *L98*). These early results already suggested that, following a misincorporation, the subsequent processing of the mismatch at the 3′ terminus is a major determinant of fidelity, with extension of the mismatch fixing the mutation and exonucle-

olytic removal of the terminal base eliminating it. In this model, antimutators would have a greater probability of degrading the terminal mismatch, whereas mutator polymerases would have a reduced probability of doing so. Studies with the base analogue 2-aminopurine (Clayton *et al.* 1979) confirmed and extended this model by showing that the misinsertion rate of 2-aminopurine was similar for three polymerases tested (wild-type, mutator, and antimutator) but that the three polymerases differed in the extent to which the 2-aminopurine residue was retained in the DNA.

Other, more recent studies have attempted to address the mechanisms responsible for the altered exo/pol ratio in greater detail. DNA-sequencing of mutator and antimutator polymerase genes revealed that many of the T4 mutator mutations resided in the (N-terminal) exonuclease domain, consistent with reduced exonuclease activity (Reha-Krantz 1988; Stocki *et al.* 1995). In contrast, the *L141* and *L42* antimutator mutations were found to reside in the polymerase part. This location is consistent with impaired polymerase activity, thus affecting the pol/exo ratio from the opposite direction as the mutators. Spacciapoli and Nossal (1994a,b) investigated in detail the *L141* polymerase (A737V) and found that the exonuclease activity as measured on single-stranded DNA was unaltered. However, on double-stranded DNA the exonuclease activity was more processive than the wild-type enzyme, while the polymerase activity was found to be significantly less processive than the wild-type enzyme. It was proposed that the antimutator polymerase somehow partitions differently between the polymerase site and the exonuclease site on the molecule, such that the possibility of finding the mismatch in the exo site is significantly increased. A defect in polymerase translocation to the next template site following incorporation was suggested as one possible mechanism by which this could occur. Similar conclusions were reached by Reha-Krantz and co-workers (Reha-Krantz and Nonay 1994; Stocki *et al.* 1995) who used elegant genetic techniques to select new mutator and antimutator mutants which have been proposed to be affected, either directly or indirectly, in the switching of the polymerase between the pol and exo sites.

Possible explanations for the observed specificity of the T4 antimutators: The model for T4 antimutator mutators in terms of altered partitioning between the exonuclease and the polymerase sites is attractive and supported by the experimental approaches. However, the model does not provide an obvious explanation for why the antimutator effect is observed only for A·T→G·C transitions and frameshifts in runs. Enhanced mismatch removal has been observed in the case of many different mismatches, including transversion mismatches (Muzyczka *et al.* 1972) and the partitioning model predicts that all types of errors should be subject to the antimutator effect. Only one paper has addressed this question in detail (Reha-Krantz 1995). She proposed that a hierarchy exists among the various possible mispairs in terms of their ability to be extended by the polymerase. Those mispairs that are most easily extended will escape the proofreader most frequently, whereas the ones that are more difficult to extend will escape less frequently. Reha-Krantz proposed that the mispairs responsible for A·T→G·C transitions (A·C and/or T·G, the template base stated first) are incompletely proofread due to their facile extension and are therefore most susceptible to increased exonuclease action, yielding a strong antimutator effect. However, in order to explain the absence of antimutator effects for all other mismatches (including the reciprocal C·A and G·T mispairs) one would have to assume that these mismatches are already maximally proofread and further proofreading enhancement would not lead to a reduction in the corresponding mutation frequency.

As an alternative, I offer the following explanation. The basic assumption is that as on the *E. coli* chromosome, mutations in T4 likely originate from various sources, of which DNA replication errors are only one (see Figure 1). (In this context, I define DNA replication errors as those resulting from the intrinsic inaccuracy of the replication of perfect, *i.e.*, undamaged, DNA templates.) Because the ability of T4 to repair its DNA is limited and T4 does not use host repair functions (Santos and Drake 1994), we suppose that a significant proportion of spontaneous mutations in T4 may actually result from the presence of low levels of DNA damage. For example, the G·C→A·T transitions might in majority result from deaminated cytosines (hydroxymethyl C in the case of T4). In that case, the absence of an antimutator effect for G·C→A·T transitions would be readily explained. Likewise, most transversions in T4 might result from DNA damages, at which increased proofreading may not significantly affect the resulting mutation rates. In contrast, A·T→G·C are the most frequent replication errors (Schaaper and Dunn 1991; Schaaper 1993a) and they may be the sole component of the spectrum of spontaneous T4 mutations resulting exclusively or predominantly from replication errors. Increased proofreading would therefore be expected to strongly affect the observed mutation frequencies. This model would also be consistent with the ability of the T4 antimutator mutants to reduce G·C→A·T transitions induced by base analogues (Drake *et al.* 1966). The analogue-induced G·C→A·T transitions would now clearly be replication errors.

Next: For more than three decades, the T4 antimutator system has served as a highly useful model system for understanding the factors involved in DNA replication fidelity. It is likely that this will continue in the near future. Recently (Wang *et al.* 1997), the crystal structure of the DNA polymerase from phage RB69, a close relative of T4, was reported. The availability of this structure is allowing the positioning of the antimutator (and mutator) mutations within the resolved molecule, facilitat-

ing the assignments of specific functions that may be affected by the mutations (see articles by Reha-Krantz and Nossal, this issue). In addition, detailed kinetic studies have been performed with the T4 polymerase (Capson *et al.* 1992), leading to the identification and quantitation of many steps in the polymerization cycle. Application of these methods to antimutator mutants should allow the proposed models for altered partitioning between the various states, including the polymerization and exonuclease states, to be tested. It is likely that the continued study of the T4 antimutators will provide increased insight into the mechanisms of replication fidelity.

B. Antimutators in *E. coli*

Antimutator mutants have also been studied in the bacterium *E. coli*. Below, I will review the three major efforts, spanning three different decades, to isolate antimutator mutants in this organism. In each case, the specific purpose was to use such antimutator mutants to probe the process of spontaneous mutagenesis in this organism. Earlier, sporadic reports on antimutator effects that were not pursued in any detail have already been briefly reviewed (see Geiger and Speyer 1977; Drake 1993).

The *E. coli mud* strain: Geiger and Speyer (1977), in a deliberate search for antimutator mutants in *E. coli*, reported on a temperature-sensitive *purB* mutant that did not grow at 42° without added adenine. At permissive or semipermissive temperatures in the absence of adenine, strong antimutator effects were observed (~500-fold for the production of valine-resistant mutants at 30°) and they called the strain *mud* (mutation defective). The antimutator effect increased with increasing temperature and was abolished at all temperatures by the addition of adenine or adenosine. These (and other) observations clearly linked the magnitude of the antimutator effect with the level of the adenine deficiency. Valine-resistance mutants appeared to be most strongly reduced, although significant effects were also observed for *met*, *his* or *lac* reversions. Interestingly, no effect was observed on the level of rifampicin- or T7-resistant mutants. The possible mechanisms underlying the *mud* antimutator were investigated in detail by Lyons *et al.* (1985), but few clues were uncovered. No interactions were uncovered between *mud* and either the inducible SOS response or the adaptive response. Also, DNA adenine methylation was normal, eliminating the possibility that an extended window for postreplicative mismatch repair (caused by delayed methylation) might lead to enhanced mismatch correction. However, the dNTP pools in a *mud* strain were found to be disturbed, leading to lower dATP, dGTP, and dTTP levels, and higher dCTP levels. While dNTP disturbances can clearly affect mutagenesis as judged from *in vitro* pool bias experiments (*e.g.*, Kunkel *et al.* 1981), it was not clear whether these observed dNTP changes were actually responsible for the *mud* antimutator phenotype.

An alternative explanation for the Mud phenotype flows from unpublished data from our laboratory. When the *mud* mutation was transferred to the strain background normally used in our laboratory, it was noticed that the appearance of valine-resistant mutants on valine-selection plates in *mud* strains was greatly suppressed on days 2 and 3 but reached normal levels on days 4, 5, and 6. The presence of adenine in the valine plates made the mutants appear normally on day 2. Thus, it appeared that in the absence of adenine, valine-resistant mutants in a *mud* strain had a growth impairment that delayed their appearance. This delay was confirmed in reconstruction experiments in which purified valine-resistant mutants selected from the *mud* strain at day 4 were mixed with normal *mud* cultures. These added, preexisting mutants appeared on day 2 in the presence of adenine but as very small colonies on day 3 that did not reach normal size until day 4 in its absence. Similar experiments performed in the AB1976 background used in the experiments of Geiger and Speyer (1977) and Lyons *et al.* (1985) suggested that the growth impairment of valine-resistant mutants in the *mud* background in the absence of adenine is even more severe than in our strain background, essentially preventing their appearance during the course of a normal experiment. Thus, if our interpretation is correct, *mud* may not be considered a true antimutator but rather a strain in which the growth of preexisting mutants on the selective medium plates is delayed or even prevented.

The Quiñones and Piechocki antimutators: Quiñones and Piechocki (1985) screened 500,000 mutagenized colonies of strain AB1976 for mutants displaying reduced papillation on EMB lactose plates containing the mutagen 2-aminopurine (2AP) (*i.e.*, a screen for 2AP-nonmutable strains). Among 70 candidates, a total of 20 were found to also display reduced mutagenesis in the absence of 2AP using two forward assays (valine resistance and 6-azauracil resistance), thus providing 20 possible spontaneous antimutators. Subsequent mapping of 11 antimutators placed them at 10 different loci on the *E. coli* chromosome. Based on their various properties, the antimutators were divided in three main groups: (1) one antimutator with enhanced replication fidelity, (2) mutants deficient in various (presumed error-prone) DNA repair pathways, and (3) auxotrophs, such as *pur*, *ser* or *thr* with presumed lower levels of metabolically induced lesions. Unfortunately, this potentially valuable set of strains has not been investigated further.

Intriguing aspects of this data set are that: (1) so many different loci on the *E. coli* chromosome appear to control spontaneous mutability, and (2) the reduction in spontaneous mutability (as measured by forward assays) by many of the antimutator alleles is very large (up to 50-fold). These findings are difficult to reconcile with the model that spontaneous mutations are a mix-

ture of largely independent sources. Thus, if one pathway contributes 90% of the spontaneous mutations, a maximally 10-fold reduction can be expected for antimutators that operate within this particular pathway. At the same time, this would preclude finding antimutators in any of the other pathways. Thus, in order to reconcile all the Quiñones and Piechocki data one would have to assume that either all antimutators that they isolated work in the same pathway and that this pathway is responsible for up to 98% of all spontaneous mutations (based on a 50-fold reduction) or that a significant overlap exists between the various pathways, such that numerous genes are responsible for mutations emanating through various pathways (largely eliminating the concept of independent pathways). As an alternative, it must be considered that at least some of the mutants are only apparent antimutators, as described for the *mud* mutation above.

***E. coli* antimutators with increased DNA replication fidelity:** In view of the possibility that in *E. coli* multiple, parallel pathways might be contributing to spontaneous mutations, Fijalkowska *et al.* (1993) undertook a different approach for obtaining antimutator mutants. Instead of isolating overall antimutators, they focused on a single pathway, in this case DNA replication errors. Their strategy was to first isolate mutants that replicate their DNA with increased accuracy (*i.e.*, antimutators for DNA replication errors). Once established, these strains could then be used to probe the question of the role of DNA replication errors among overall spontaneous mutations.

Antimutators in the DNA replication pathway were obtained as suppressors of the high mutability of a mismatch-repair-defective *mutL* strain, using a papillation assay on MacConkeyGal plates (Fijalkowska *et al.* 1993). Localized mutagenesis was performed of the *dnaE-dnaQ* region of the *E. coli* chromosome, because these two genes are located near to each other in the 4 to 5 min region of the chromosome and were considered the primary target for replication-specific antimutators. The two genes encode the polymerase (*dnaE*) and the proofreading (*dnaQ*) activity of DNA polymerase III holoenzyme that performs the replication of the *E. coli* chromosome. In contrast to the T4 DNA polymerase, the *E. coli* polymerase and the proofreading exonuclease are contained in different polypeptides, although the two subunits are found tightly bound together in the pol III core (Kelman and O'Donnell 1995). Among a total of 20,000 mutagenized colonies in two different experiments (Fijalkowska *et al.* 1993; J.-Y. Mo and R. Schaaper, unpublished results), a total of 13 isolates have been obtained that reproducibly reduced the papillation level. Subsequent direct measurement of mutant frequencies using several different mutational markers confirmed the suppression of the *mutL* mutator phenotype. The antimutator effect depended on the *dnaE* allele and the mutational marker scored but varied between 2- and 30-fold (Fijalkowska *et al.* 1993). Mapping and DNA sequencing showed each of them to have a single, but different, amino acid substitution in the *dnaE* gene (Fijalkowska and Schaaper 1993; J.-Y. Mo and R. Schaaper, unpublished results), consistent with the presumed increased DNA replication accuracy. The colony size and growth rate of the mutants were very similar to those of the wild-type $dnaE^+$ strain, making it unlikely that the effect resulted from an inability to express the mutations.

To see if the *dnaE* alleles also conferred an antimutagenic effect in the mismatch-repair-proficient $mutL^+$ background, the alleles were transferred into a wild-type background. Careful measurement of mutant frequencies revealed an approximate twofold reduction in rifampicin-resistant mutants or forward *lacI* mutants (Oller and Schaaper 1994). The implication of this finding is that about 50% of the spontaneous mutations in growing *E. coli* cultures may be ascribed to uncorrected DNA replication errors. This percentage could be greater if the *dnaE* alleles would be capable of reducing, for example, only a certain fraction or only certain classes from among all replication errors. The specific effect of the antimutator alleles was further investigated by DNA sequencing spectra of *lacI* forward mutations in $dnaE^+$ (wild-type) and *dnaE911* (antimutator) strains. The results showed that from the many mutational classes that are present in a spontaneous spectrum (Schaaper and Dunn 1991) only the base substitutions were significantly reduced by the *dnaE911* allele, and among these only the transversions were reduced (Oller and Schaaper 1994). The fact that the *dnaE911* allele specifically removed a subfraction of the overall mutations is convincing evidence that our *dnaE* antimutators are true antimutators and not impaired mutants that have difficulty displaying mutations.

Importantly, these studies identify that the spontaneous transversion mutations result from DNA replication errors. Conversely, the spontaneous transitions (mostly G·C→A·T) must be argued to arise from a different source, *e.g.*, DNA damage-related events, such as cytosine deamination, alkylguanine, oxidative damages, *etc.* The lack of antimutator effect for the transition mutations does not result from the inability of the antimutator alleles to reduce these types of errors, because transitions are effectively reduced in a mismatch-repair-defective background (Fijalkowska *et al.* 1993; Schaaper 1993b). That spontaneous transversion mutations represent replication errors, while spontaneous transition mutations may be DNA damage-induced events, is perhaps counterintuitive (as many DNA-damaging treatments tend to cause transversions) but can be understood in the context of the specificity of *mutHLS*-dependent DNA mismatch repair. This repair system corrects transitions much more efficiently than transversions (*e.g.*, Schaaper 1993a). Thus, among errors of DNA replication, the transitions are very well corrected, essentially removing

them from the spectrum of spontaneous mutations or, at least, bringing them down below the level of transition mutations emanating from other pathways. On the other hand, transversion replication errors are only weakly corrected, leaving them above the level of transversions produced by other pathways. Thus, a *dnaE*-mediated reduction in transversion errors can be observed in the mismatch-repair-proficient strain but a reduction in transition errors cannot. Our current efforts are to delineate more precisely the source of the spontaneous mutations that are not subject to the *dnaE* antimutator effects.

One important qualification to our conclusions relates to the possibility that the *dnaE* antimutator alleles not only reduce normal DNA replication errors but also reduce mutations at DNA lesions. This is currently being investigated. Results obtained so far suggest that the *dnaE* antimutator alleles are effective in reducing mutations in *mutT* backgrounds, *i.e.*, they prevent $A_{template}$·(8-oxodGTP) mispairings (Schaaper 1996), as well as mutations induced by certain base analogues (Pavlov *et al.* 1996; Schaaper and Dunn 1997). Thus, the possibility that the decrease in spontaneous mutations in wild-type backgrounds results, at least in part, from mutations occurring at DNA mispairing lesions must be left open. In contrast, the *dnaE* antimutator alleles are totally ineffective in reducing mutagenesis proceeding through the SOS pathway (Fijalkowska *et al.* 1997), thus eliminating the class of "bulky" or "noncoding" DNA lesions from consideration. Interestingly, the *dnaE915* antimutator allele was shown to be highly effective in reducing certain mutations in stationary cells (sometimes called adaptive mutations) (Foster *et al.* 1995; Harris *et al.* 1997), implicating DNA polymerase III in generating these mutations.

In addition to probing the sources of spontaneous mutations, the *dnaE* antimutators may also be useful for understanding the mechanisms of DNA replication fidelity in *E. coli*. Data from our laboratory (J.-Y. Mo and R. Schaaper, unpublished results) measuring the insertion fidelity of purified α subunit from wild-type and the *dnaE911* antimutator polymerase indicate that there is no difference between the two polymerases in base insertion fidelity. Thus, the antimutator effect is likely to be exerted in a indirect manner. For example, as in the case of the T4 antimutators, the efficiency of the proofreading step may be enhanced because of impaired extension from mismatched primer termini. However, in addition to this mechanism, an additional pathway must also operate, because we observed that the *dnaE* antimutator alleles are also highly effective in reducing the mutation rate in proofreading-defective *mutD5* and *dnaQ926* strains (Fijalkowska and Schaaper 1995, 1996). We have suggested that in this background enhanced fidelity is achieved by increased dissociation of DNA polymerase from terminal mismatches, providing an opportunity for removal of the terminal base by alternative means, such as other 3′ exonucleases, either free or polymerase-associated.

Perspectives: The approach described above for gaining insight into spontaneous mutagenesis through pathway-specific antimutators can be readily expanded. For example, whether unrepaired DNA uracils (resulting from cytosine deamination) contribute to the frequent occurrence of spontaneous G·C→A·T transitions, which are not susceptible to *dnaE* antimutator effects (see above), may be addressed by isolating or creating mutants overexpressing the enzyme DNA uracil-glycosylase, followed by measurement of the effect of such mutations on spontaneous mutagenesis. As another example, the role of oxidative DNA damage in spontaneous mutagenesis could be investigated through mutants with reduced mutability by oxidative agents. Such analysis, using a collection of pathway-specific antimutator mutants, should provide a comprehensive picture of the pathways responsible for spontaneous mutagenesis in *E. coli*. It will be of interest to extend such analyses to cells growing under a variety of different conditions, including stationary phase in which mutations may arise by different combinations of pathways. Finally, this approach may provide an important paradigm for studies of spontaneous mutagenesis in other organisms.

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