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Patch Length of Localized Repair Events: Role of DNA Polymerase I in *mutY*-Dependent Mismatch Repair

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In vivo experiments with heteroduplex lambda genomes show that the MutY mismatch repair system of *Escherichia coli* defines an average repair tract that is shorter than 27 nucleotides and longer than 9 nucleotides and extends 3′ from the corrected adenine. The phenotype of a mutant defective in DNA polymerase I shows that this enzyme plays a significant, though not an essential, role in the in vivo repair of apurinic sites generated by this system. Evidence is presented that in the absence of polymerase I the repair tracts are modestly longer than in the *polA*$^+$ extending in the 5′ direction from the corrected adenine, suggesting a role for another DNA polymerase.

The systems responsible for the repair of base pair mismatches in DNA can be divided into two groups: (i) those in which long excision repair tracts are associated with the correction, and (ii) those responsible for localized repair. The methyl-directed system in *Escherichia coli* (20), the hex pathway in *Streptococcus pneumoniae* (5, 24), and possibly the nick-directed repair in eucaryotic cells (20) belong to the first group. The group of localized repair systems includes the very short patch (15) and MutY (1, 16, 21) repair systems of *E. coli*, the *mutB*-dependent repair in *Salmonella typhimurium* (17), the G/T-to-G/C short patch repair in mammalian cells (30), and possibly the G/A repair that is evident in *S. pneumoniae* (22) and in mammalian cells (32).

The MutY pathway of *E. coli* acts on C/A (21) and G/A mismatches (1, 21) and plays a role in a mutation avoidance system that acts to eliminate the consequences of the presence of 8-hydroxyguanine in DNA (18). A single gene, *mutY* (*micA*) (19, 27), has been so far positively identified as encoding a product required for adenine correction. The mechanism suggested for this pathway postulates the removal of the mispaired adenines of G/A and C/A mismatches and nicking of the first phosphodiester bond 3′ to the apurinic site (26). The MutY product has been reported to possess the N-glycosylase and 3′ apurinic endonuclease activities capable of performing the steps described above (26), although the endonuclease activity was not evident in the preparation described by Au et al. (2). The subsequent steps to complete the repair require the action of one or more nucleases, cleaving at least the second phosphodiester bond 5′ of the nick to remove the deoxyribose phosphate (15), followed by opening of the gap and then resynthesis and ligation of the gapped strand. This pathway may well be a common one for all apurinic lesions. The aims of this work were to define the length of the repair tracts and to identify the functional requirements for the repair other than the product of the *mutY* gene.

Repair tracts. To determine the lengths of tracts in the MutY initiated repair events in vivo, Su$^-$ *mutL* mutant cells were infected with λ phages containing packaged heteroduplex genomes. The genomes were products of annealing separated complementary strands, each obtained from phages carrying a different amber mutation in the *P* or *O* genes (Table 1), whose products are both required for the initiation of phage replication. In order to obtain an infective center on a Su$^-$ strain, the mutant nucleotide on the transcribed strand (A opposite G or C) must be replaced. Furthermore, the tract of this repair process must be short enough to avoid cocorrection of the neighboring wild-type nucleotide that is mismatched with a mutant nucleotide present in the complementary strand. This last feature permits the evaluation of the length of the repair tract by determining the efficiencies of plating of various heteroduplex phages on the Su$^-$ indicators. The mutation in the *mutL* gene is present to disable the methyl-directed and very short patch mismatch repair systems. Strain PR123 also harbors a plasmid, pJTW4-20D601, encoding the MutY product (27), thus enhancing the efficiency of the repair of C/A and G/A mismatches. The results are displayed in Table 2. The low yield of P^+ bursts reported in the column corresponding to the double mutant strain indicates that basically all the correction detected in the other two strains is performed through the MutY system. With complementary strand markers as little as 9 nucleotides 5′ from the mutant A, no significant effect on the yield of P^+ phage is evident (heteroduplexes i and ii). Conversely, when the mutant marker on the complementary strand is 3′ from the mutant A on the transcribed strand, its presence at 9 nucleotides from the A results in a reduction of the yield of P^+ phage (heteroduplex iii). If the marker in this orientation is 27 bases away, the yield of P^+ phage is unaffected (heteroduplex iii). It appears that the average repair tract in the 3′ direction from the excised adenine is longer than 9 but shorter than 27 nucleotides. Furthermore, the gap expands less than 9 nucleotides in the 5′ direction from the excised adenine. This is consistent with in vitro data that show that the average replacement patch in the MutY-dependent pathway is shorter than 12 nucleotides (25).

Isolation of mutants. We have applied the same genetic screen used for the detection of the *micA* (*mutY*) locus to isolate mutants affecting the excision gap in the repair of C/A mismatches (21). Briefly, PR9 (M182 Su$^-$ *galU galK lacZX74* Sm^r *mutL*::Tn*10*) cells were mutagenized by infection with a λ phage carrying a modified Tn*10* (mini-Tn*10* Kan^r) (28), incubated for 1 h at 37°C, and spread on agar plates containing kanamycin (0.05 mg/ml) along with ~10^6 randomly reconstituted heteroduplex-containing λ phages, *c*I60*P*am3/*c*I60*P*am80. The amber mutations on the complementary strands are separated by 27 nucleotides, and the *mutL* phenotype of the bacterial host results in the avoidance of the loss of heteroduplex substrates by the methyl-directed mismatch re-

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TABLE 1. Bacteriophage λ strains

Mutation[a]	Mutation position[b]	Base change
O905[c]	38797	G·C to T·A
O29[c]	38914	G·C to T·A
P2056[d]	39657	C·G to T·A
P574[d]	39675	C·G to T·A
P584[d]	39684	C·G to T·A
P573[d]	39717	C·G to T·A
P80[c]	39759	C·G to T·A
P3[c]	39786	C·G to T·A
P575[d]	39831	C·G to T·A
P567[d]	39864	C·G to T·A
P559[d]	39894	C·G to T·A

[a] All mutants have the A on the transcribed strand of λ.
[b] Number of the nucleotide in the λ sequence.
[c] See reference 20.
[d] The phage strain is from Ira Herskowitz. The *P* mutation was sequenced in this laboratory.

pair. As the bacterial colonies grow they encounter heteroduplex-containing phage. Localized correction of the mutant A of the transcribed strand in the C/A mismatch removes the amber mutation in the *P* gene that encodes an essential replication function, leading to the lysis of the bacteria and giving rise to colonies with edges nibbled by the *c*I60 P^+ phages that result. Colonies that failed to display nibbled edges were candidates for the mutants we were seeking. This screen yielded six mutant strains that showed a reduction of the plating efficiency of the heteroduplex phage without affecting that of λ. Five of the mutants isolated could be complemented by the expression of MutY from the plasmid pJTW4-21, which carries a functional *mutY* gene (27), and were presumably mutant in the *mutY* gene. The sixth mutant displayed a modestly reduced (36%) capacity to form P^+ infective centers with packaged heteroduplex *P*am3/*P*am80 phage. We designated this strain PR133.

Characterization of the mutant. We found that we were unable to introduce the MutY-producing plasmid pJTW4-21 into the PR133 strain. All 10 kanamycin-resistant products of a P1 transduction from the mutant strain into PR9 (*mutL* Su$^-$) displayed both the reduction of plating efficiency of the *P*am3/*P*am80 heteroduplex phage and the failure of plasmid transformation.

The location of the mutation on the physical map of *E. coli* was determined by cloning the mutant allele and using the cloned sequence as a probe on a filter containing the ordered Kohara λ library of *E. coli* W3110 (11). The chromosomal DNA of PR133 was digested with *Bgl*II, which does not cut within the mini-Tn*10*. The products of the digestion were ligated into the *Bam*HI site of the plasmid pTZ19U (United States Biochemical) and introduced by transformation into M182 (Su$^-$ *galU galK lacZ*Δ*X74* Sm^r) competent cells. After selection on Luria-Bertani plates containing ampicillin (0.1 mg/ml) and kanamycin (0.05 mg/ml), the colonies obtained harbored a plasmid with an ~12-kb insert. The plasmid DNA was extracted and a 1.5-kb *Acc*I-*Sac*I fragment adjacent to the mini-Tn*10* insertion was purified through low-melting-point agarose. This fragment was labeled with [α-^{32}P]dCTP by random hexamer priming (9) and used as a probe on the filter containing the λ library of *E. coli* (11). The probe hybridized to phages 547 and 548 of the Kohara miniset, indicating that the mutation is located near min 86 of the *E. coli* chromosome (12). This location coincides with the position of the *polA* gene, coding for the DNA polymerase I (Pol I). To investigate the possibility that the mutation was a *polA* allele, we tested the ability of PR133 to support the replication of other ColE1 plasmids. We were indeed unable to obtain transformants with plasmids like pACYC184, pBR322, or pK600, all containing ColE1-related origins which require Pol I for initiation of replication (3, 13). On the other hand, PR133 could be transformed with pSC101 (6), a plasmid that does not require Pol I for its replication. Furthermore, PR133 proved to be UV-sensitive. Restriction analysis of the cloned genomic frag-

TABLE 2. Efficiency of plating of heteroduplex phages on Su$^-$ *polA*$^+$ strains

Heteroduplex[a]	T* mutant	No. of bp between markers	% of heteroduplex genomes giving rise to P^+ bursts		
			mutL[b]	*mutL* pMutY[c]	*mutL mutY*[d]
i -----C----------T*-----3′ -----A*---------G------5′ *P574*	*P584* *P3* *P567*	9 111 189	6.8 7.5 8.1	55 56 54	1.5 1.2 1.2
ii -----C----------T*-----3′ -----A*---------G------5′ *P2056*	*P574* *P584* *P3*	18 27 129	20 22 23	59 56 57	0.5 1.6 0.6
iii -----T*---------C------3′ -----G----------A*-----5′ *P584*	*P574* *P2056* *O29*[e]	9 27 770	1.8 7.2 6.9	18 54 56	0.3 1.6 2.0
iv -----C----------T*----3′ -----A*---------G------5′ *P584*	*P80* *P567*	75 180	8.2 9.0	52 55	1.4 0.6

[a] Asterisks indicate the mutant nucleotides.
[b] Efficiency of plating of the heteroduplexes on PR9 (Su$^-$ *mutL*).
[c] Efficiency of plating of the heteroduplexes on PR123 (Su$^-$ *mutL* pJTW4-20 D601).
[d] Efficiency of plating of the heteroduplexes on PR68 (Su$^-$ *mutL mutY*).
[e] Creates a C/T* mismatch.

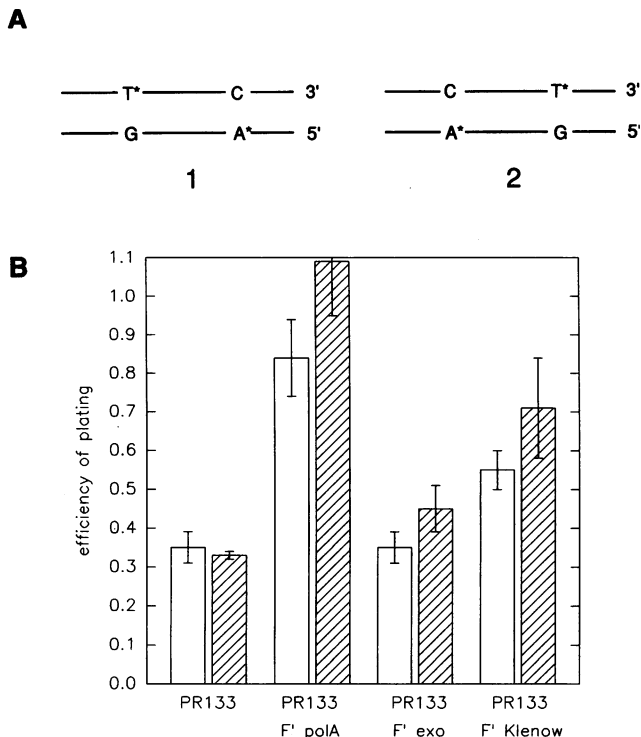

FIG. 1. Relative plating efficiencies of heteroduplex-containing phages on different strains. (A) The two possible configurations of the mismatches present in the heteroduplexes tested. The asterisks indicate the mutant nucleotides. In order to obtain a burst, the mutant A must be corrected without cocorrection of the wild-type G. (B) Plating efficiencies of the heteroduplex phages on derivatives of PR133 (Su^- *mutL polA133*) carrying episomes with the indicated portion of the *polA* gene (10). In all cases the mismatches are 27 bp apart. For each orientation two different sets of markers in the *P* gene were analyzed. The efficiencies are expressed as the fraction of the plating efficiency on PR9 (Su^- *mutL*). Each value represents the average for the two sets of markers. Open and hatched bars correspond to the heteroduplex configurations 1 and 2 in panel A, respectively.

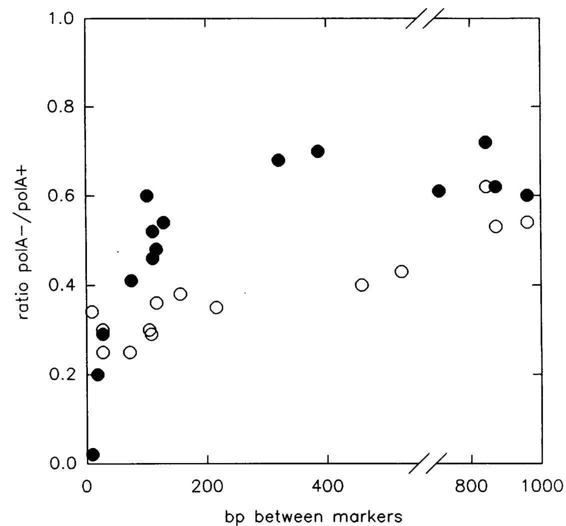

FIG. 2. Efficiency of plating of λ phages containing heteroduplex genomes on $polA^+$ (PR208) and *polA* Su^- (PR209) strains. In order to give a burst, all the heteroduplexes tested require the correction of the mutant adenine on the transcribed strand for the *O* or the *P* genes. For each heteroduplex a second amber mutation is present in the complementary strand, creating another mismatch at different distances from the G/A or C/A mismatch. Open and closed circles correspond to cases in which the second mismatch (G/T or C/T) is located 3′ (Fig. 1A, configuration 1) or 5′ (Fig. 1A, configuration 2) to the mutant adenine, respectively.

ment located the transposon ~1 kb from the start of transcription of *polA*, in the segment coding for the 5′-3′ exonuclease activity (10).

The identity of the mutation in PR133 as a defective allele of *polA* was confirmed by introducing, through conjugation, F′ episomes carrying the *polA* gene or the sequences expressing either the polymerase-3′-5′ exonuclease or the 5′-3′ exonuclease activities (10). Only the episome carrying the intact *polA* gene was able to restore the UV resistance and the ColE1 plasmid maintenance proficiency in PR133, suggesting that both Pol I activities are impaired in the mutant. The plating efficiency of the test heteroduplex-containing phage in the episome-carrying derivatives of PR133 suggests that the expression of the Klenow fragment results in partial restoration of the localized repair (Fig. 1).

To determine the in vivo effect of the *polA133* mutation on the MutY-dependent repair of mismatches, PR133 cells were infected with λ phages harboring packaged heteroduplex genomes. To facilitate the analysis of the effect of the *polA* mutation on mismatches that are poorly corrected by this system (4), the *mutY* gene from pJTW4-21, cloned into pSC101, was introduced into the *polA* strains. This allows the overexpression of MutY in *polA* strains. For this purpose, a 2-kb *Fsp*I-*Sal*I fragment from pJTW4-21, carrying the complete *mutY* gene, was cloned between the *Xho*I and *Pvu*II sites of pSC101, creating pMUTY7. This plasmid was introduced into PR138, a tetracycline-sensitive derivative of PR9, and into PR198, the *polA133* version of PR138, to create PR208 and PR209, respectively. The enhanced production of MutY increases the efficiency of repair of all the mismatches, with the possible exception of the G/A from the *O29* mutant, which experiences 60 to 80% correction in the parent strain PR9 (21). The heteroduplex phages, constructed with separated DNA strands of the various mutants and annealed in different combinations, were then plated on PR208 (Su^- *mutL* pMUTY7) and PR209 (Su^- *mutL polA133* pMUTY7). The efficiency of formation of infective centers was then evaluated. In order to analyze the impact of distance between the markers, the ratio of the efficiency of P^+ phage formation in the *polA* mutant (PR209) to that in the $polA^+$ (PR208) strain was calculated for each pair of markers. Context effects on the repair of the various markers (4, 21) are thus eliminated. Figure 2 shows the plot of the ratio as a function of the distance in base pairs between the markers in the heteroduplex phage. Open circles represent the relative efficiency of correction when the mutant marker on the nontranscribed strand is 3′ to the mutant A in the transcribed strand (configuration 1 in Fig. 1A). In this case the distance between markers seems to have little or perhaps no effect on the efficiency of the localized repair. Closed circles indicate that the mutant marker in the complementary strand is 5′ to the required A correction (configuration 2 in Fig. 1A). For the latter case, there is a marked decrease in the relative ability of the *polA* mutant strain to yield wild-type phage when the second mismatch is close to the G/A or C/A one. Thus, a prominent effect of the loss of Pol I activity appears to be the expansion of the excision repair tract in the 5′ direction from the removed A. The data

in Fig. 2 also display an overall reduction in the yield of wild-type products, independent of the orientation and of the distance between the mutant nucleotides. It is possible that this reflects some residual activity of the very short patch system, acting on the complementary strand. However, when phages containing single G/A or C/A mismatches were plated on the *polA* mutant strain, a modest reduction (15 to 40%) of the efficiency of plating was observed, suggesting a modest reduction of the viability of the products of MutY action when *polA* is disabled.

Role of polymerases in mismatch repair. From these observations, we conclude that Pol I plays a prominent, though not essential, role in the completion of the *mutY*-dependent localized repair of G/A and C/A mismatches, providing in vivo confirmation of the in vitro experiments with *polA* mutant strains (25, 29) and of the postulated involvement of DNA Pol I in the repair of apurinic sites in general. In addition, a similar role for Pol I appears evident in the very short patch pathway (8). In the case of mammalian cells, the effective repair of G/T mismatches requires the activity of Pol β, functionally related to Pol I from *E. coli* (31).

We have shown that in the absence of either of the Pol I functions, the DNA polymerase, or the 5′-to-3′ exonuclease, localized correction can take place but the yield of P^+ products is reduced. In the absence of Pol I another polymerase could be carrying out the resynthesis step of the repair. It has been shown that in the case of UV damage, repair in *polA*-defective mutants occurs with a reduced efficiency but the de novo DNA synthesis is more extensive than in the *polA*-proficient cells (7). Presumably Pol II and/or Pol III can substitute for Pol I, but larger gaps accompany their action (13). The gap expansion could be performed by other exonucleases and/or by strand displacement. The modest impact of distance when the complementary strand mutant base is 3′ to the mutant adenine (Fig. 2) suggests that short patches of repair occur in the absence of Pol I. Thus there appears to be a polymerase with low processivity, perhaps Pol II, that can substitute for Pol I without a significant change of the repair tract length in the 3′ direction, either by exonuclease activity or by strand displacement. When the complementary strand mutant nucleotide is 5′ to the mutant adenine (Fig. 2) the prominent reduction in yield at short distances suggests more extensive gap expansion by the action of 3′-to-5′ exonuclease when Pol I is absent. Therefore, the use of the alternate polymerase seems to be accompanied by more 3′-to-5′ digestion prior to the onset of the synthesis step. The overall reduction in the yield of P^+ products when there are large distances between markers in either orientation, or when a single mismatch is present in the heteroduplex, could be due to the reduced viability of products associated with the alternate repair process. Our findings are in agreement with the proposal that DNA Pol I plays a prominent, though not essential, role in localized repair systems.

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