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Competition between MutY and Mismatch Repair at A · C Mispairs In Vivo

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We show that the MutY protein competes with the MutS-dependent mismatch repair system to process at least some A · C mispairs in vivo, converting them to G · C pairs. In the presence of an increased dCTP pool resulting from the loss of nucleotide diphosphate kinase, the frequency of A · T→G · C transitions at a hot spot in the *rpoB* gene is 30-fold lower in a MutY-deficient derivative than in the wild type.

The MutY protein, the product of the *mutY* gene in *Escherichia coli* (18), is a glycosylase that plays an important role in the repair of oxidatively damaged DNA (11, 12). Loss of function in both chromosomal copies of the human gene encoding MutY leads to increased susceptibility to colon cancer in humans (1, 9). MutY removes A residues that are mispaired with 7,8-dihydro-8-oxoguanine (oxoG) (11, 13), a frequent oxidation product of DNA (4). This removal allows repair polymerases to restore a C across from the 8-oxoG (25), allowing the MutM protein to remove the 8-oxoG (11, 13; for reviews, see references 12 and 15). Subsequent repair synthesis restores the original G · C base pair. MutY also removes the A from A · G mispairs (2), from A · 8-oxoA mispairs (13), and, to a lesser extent, from A · C mispairs (13, 21). Because the MutY protein does not discriminate between old and new strands, the ability to remove A from A · C mispairs may potentially immortalize mutations stemming from A · C mispairs in which A is the correct base, even though these mispairs may be substrates for correction by the MutSHL-dependent mismatch repair (MMR) system (for a review, see reference 17). In these cases, the original A · T base pair would be converted to a G · C base pair. In this sense, the MutY protein competes with the MMR system for the processing of A · C mispairs. Figure 1 portrays the different outcomes of A · C mispairs arising from replication errors at an A · T base pair.

The *E. coli rpoB*/Rif^r system. We decided to study the contribution of the MutY protein to A · T→G · C mutations that can arise via an A · C mispair by using the previously described *E. coli rpoB*/Rif^r system to monitor mutations (7). We have extended the work of others (8, 19, 22–24) to generate a system that can analyze 69 different base substitutions in the *rpoB* gene (7). Recent work (20; E. Wolff, M. Kim, and J. H. Miller, unpublished data) has added several sites to this collection, so that as many as 73 different base substitutions can be monitored by analyzing *E. coli* Rif^r mutants at 37°C (Table 1). Of these 73 mutations, one particular A · T→G · C mutation, at bp 1547, is a hot spot in a wild-type background and a very strong hot spot in a *mutS* background (7, 16). Although it is not

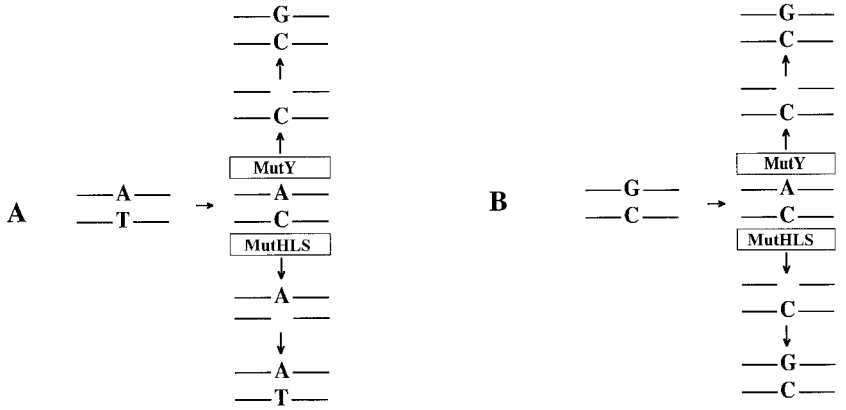

FIG. 1. Competition between the MutY protein and the MutHLS MMR system. (A) Misreplication at an A · T base pair leads to an A · C mispair that is corrected by the MutHLS MMR system but converted to a G · C pair by MutY. (B) Misreplication at a G · C base pair leads to an A · C mispair that is converted back to a G · C pair by both MutY and the MMR system.

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TABLE 1. Distribution of mutation in *rpoB*

Mutation	Site (bp)	Amino-acid change	No. of strains with *rpoB* mutation					
			WT	*mutS* mutant	*mutS mutY* double mutant	*mutY* mutant	*ndk* mutant	*ndk mutY* double mutant
AT → GC	443	Q148R	5	0	0	0	1	0
	1522	S508P	0	0	0	0	0	0
	1532	L511P	2	7	7	0	2	0
	1534	S512P	9	11	7	0	2	3
	1538	Q513R	0	3	0	0	1	0
	1547	D516G	23	48	17	1	70	2
	1552	N518D	1	3	4	0	0	0
	1577	H526R	0	0	0	0	0	0
	1598	L533P	0	0	0	0	0	0
	1703[a]	N568S	0	0	0	0	0	0
	1715	I572T	0	0	0	0	0	0
GC → AT	1520	G507D	0	0	0	0	0	0
	1535	S512F	4	0	0	0	0	0
	1546	D516N	3	9	1	0	0	0
	1565	S522F	3	0	0	0	0	0
	1576	H526Y	7	0	0	1	0	0
	1585	R529C	0	0	0	0	0	0
	1586	R529H	2	1	0	0	0	0
	1592	S531F	5	1	0	0	0	0
	1595	A532V	0	0	0	0	0	0
	1600	G534S	0	3	0	0	0	0
	1601	G534D	0	0	0	0	0	0
	1610[a]	G537D	0	0	0	0	0	0
	1691	P564L	4	0	0	0	0	0
	1721	S574F	4	0	0	0	0	0
	2060[b]	R687H	0	0	0	0	0	0
AT → TA	443	Q148L	18	0	0	0	6	18
	1532	L511Q	1	0	0	0	3	0
	1538	Q513L	7	0	0	0	0	0
	1547	D516V	1	0	0	0	0	0
	1568	E523V	0	0	0	0	0	0
	1577	H526L	0	0	0	0	0	0
	1598	L533H	2	0	0	0	0	0
	1714	I572F	4	0	0	0	0	0
	1715	I572N	1	0	0	0	0	0
AT → CG	437	V146G	0	0	0	0	0	0
	443	Q148P	0	0	0	0	0	0
	1525	S509R	1	0	0	0	0	0
	1532	L511R	3	0	0	0	1	0
	1534	S512A	0	0	0	0	0	0
	1538	Q513P	0	0	0	0	0	2
	1547	D516A	0	0	0	0	0	1
	1577	H526P	1	0	0	0	0	1
	1598	L533R	0	0	0	0	0	0
	1687	T563P	0	0	0	0	0	0
	1714	I572L	14	0	0	0	1	0
	1715	I572S	4	0	0	0	2	0
GC → TA	436	V146F	0	0	1	0	0	0
	442	Q148K	0	0	0	1	0	0
	444	Q148H	1	0	0	2	0	0
	1527	S509R	2	0	0	2	0	0
	1535	S512Y	0	0	0	0	0	0
	1537	Q513K	0	0	0	0	0	0
	1546	D516Y	2	0	0	0	0	0
	1565	S522Y	0	0	0	1	0	0
	1576	H526N	2	0	0	0	0	0
	1578	H526Q	0	0	0	0	0	0
	1586	R529L	0	0	0	0	0	0
	1592	S531Y	0	0	0	2	0	0
	1595	A532E	0	0	0	0	0	0
	1600	G534C	3	0	0	2	0	0
	1601	G534V	1	0	0	1	0	0
	1708	G570C	0	0	0	0	0	0
	1721	S574Y	2	0	0	2	0	0
	1585[b]	R529S	0	0	0	0	0	0

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TABLE 1—*Continued*

Mutation	Site (bp)	Amino-acid change	No. of strains with *rpoB* mutation					
			WT	*mutS* mutant	*mutS mutY* double mutant	*mutY* mutant	*ndk* mutant	*ndk mutY* double mutant
GC → CG	444	Q148H	0	0	0	0	0	0
	1527	S509R	1	0	0	0	0	0
	1574	T525R	1	0	0	0	0	0
	1576	H526D	3	0	0	0	0	0
	1578	H526Q	0	0	0	0	0	0
	1585	R529G	0	0	0	0	0	0
	1601	G534A	1	0	0	0	0	0
	1691	P564R	0	0	0	0	0	0
	1709	G570A	0	0	0	0	0	0
	1716	I572M	0	0	0	0	0	0
None found			4	0	0	0	0	0
Total[c]			152	86	37	15	90	27

[a] Sites that have been detected in a related study (J. H. Miller, E. Wolff, and M. Kim, unpublished data).
[b] Sites that show temperature effects (8) between 30 and 42°C and that may not yield Rif^r colonies at 37°C.
[c] The results for 73 sites were considered for the total, but the number increases to 75 with the inclusion of the sites indicated by footnote *b.* Mutations in the wild-type, and the *mutS* mutant were described previously (7), as was the distribution of a smaller subset of the *ndk* mutations (16).

clear what proportion of the A · T→G · C transition mutations at this site result from A · C rather than T · G mispairs, comparing the rates of mutations at this hot spot in wild-type and *mutY* backgrounds seems to be a straightforward way to look for effects of the presence or absence of MutY on transitions. However, G · C→T · A mutations are elevated in a *mutY* background, significantly increasing the Rif^r mutant frequencies (17) (Table 2). Only a small percentage of the Rif^r mutants would be caused by other mutations. Thus, 13 of 15 mutations in *rpoB* obtained in a *mutY* strain result from G · C→T · A transversions (Table 1). Therefore, we sought to increase the level of A · C mispairs by employing a strain with a defect in the *ndk* gene (16), which encodes the enzyme nucleotide diphosphate kinase. Nucleotide diphosphate kinase is involved in maintaining nucleotide triphosphate levels, and *ndk* mutant strains have 20-fold-higher levels of dCTP and 7-fold-higher levels of dGTP, as well as higher levels of spontaneous mutations (10), than do strains without a defect in the *ndk* gene. We have analyzed the mutator effect of *ndk* strains and shown that certain base substitutions and frameshifts are considerably elevated in *ndk mutS* double mutants, indicating that replication errors are involved (16). Among mutations in *rpoB* leading to Rif^r, the mutations in both *ndk* and *ndk mutS* strains predominate at the A · T→G · C hot spot at bp 1547 (16). Because the level of mutations at this hot spot in *ndk* strains is high enough, we can determine whether the MutY protein is involved in generating A · T→G · C mutations at the bp 1547 hot spot by comparing the levels of mutations at this site in *ndk* and *ndk mutY* strains (for methods, see references 7 and 14).

Distribution of mutations in *rpoB*. Table 1 shows the distribution of *rpoB* mutations in wild-type, *mutS*, *mutY*, and *ndk* strains and in the *ndk mutY* and *mutS mutY* double mutants. Table 2 shows some comparative mutation frequencies and mutation rates per replication. *ndk* strains have 43-fold-higher rates of *rpoB* mutations that lead to Rif^r colonies than the wild type, and *mutS* strains have approximately a 80-fold-higher mutation rate (Table 2) than the wild type, although most of the mutations in the *ndk* and *mutS* strains are at one site (position 1547; A · T→G · C) (Table 1). The defect in *mutY* has no detectable effect on the mutation rate in *mutS* strains (data not shown) and no effect on the *mutS* spectrum (Table 1; note the different sample sizes). This lack of effect occurs because in the absence of MMR, which is the consequence of being a *mutS* mutant, there is no way to repair A · C mismatches, derived from A · T base pairs (Fig. 1A), regardless of the presence or absence of the MutY protein. However, in an *ndk* strain that is MMR proficient, the defect in the *mutY* gene lowers the frequency of mutations in *rpoB* 2.5- to 3-fold (Table 2) and, most importantly, virtually eliminates the position 1547 A · T → G · C hot spot from the mutational spectrum (Table 1). In the absence of this hot spot, the next most frequent mutation, A · T→T · A at position 443, now becomes the most frequent mutation and appears as a new hot spot. Figure 2 incorporates the mutation frequencies into the comparison of the *ndk* and *ndk mutY* spectra and shows that MutY-deficient derivatives of *ndk* strains have 30-fold-lower levels of A · T→G · C mutations at position 1547 in *rpoB*. (The apparent severalfold increase of A · T→T · A mutations at position 443 may not be significant, because of the small sample size of these mutations at position 443 in the distribution for the *ndk*

TABLE 2. *rpoB* frequencies and notes of mutations[a]

Strain	Mean value (95% CL)[b]	
	f (10^{-8})	μ (10^{-8})
WT[c]	7.6 (5.2–8.8)	1.5 (1.1–1.7)
mutY mutant	79 (68–90)	11 (7.4–12)
ndk mutant	570 (530–630)	64 (60–70)
mutS mutant	935 (848–1,110)	120 (110–140)
ndk mutY mutant	206 (126–280)	26 (17–34)

[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) per replication were calculated from these values by the method of Drake (5).
[b] CL, confidence limits (3).
[c] WT, wild type.

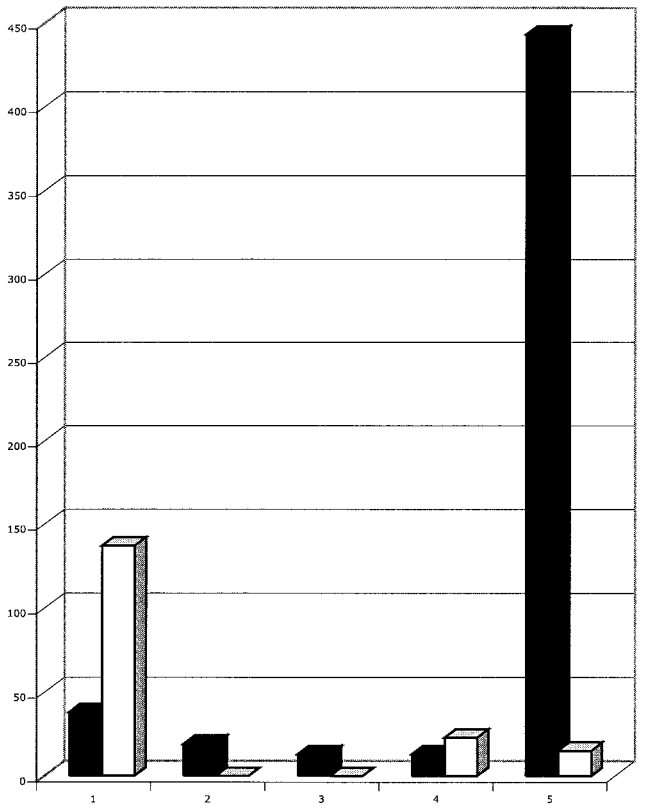

FIG. 2. Comparative mutation frequencies in *ndk* and *ndk mutY* strains. The frequencies of mutations in the *rpoB* gene at five different sites are shown for both *ndk* (white bars) and *ndk mutY* (black bars) backgrounds (see also Tables 1 and 2). The five sites (left to right) are as follows: 1, A · T→T · A at bp 443; 2, A · T→T · A at bp 1532; 3, A · →G · C at bp 1532; 4, A · T→G · C at bp 1534; 5, A · T→G · C at bp 1547.

mutants.) This finding demonstrates an in vivo effect of MutY on increasing transition mutations at certain A · T base pairs due to its ability to remove A from A · C mispairs and represents an example of a repair enzyme actually being involved in creating mutations under certain conditions. The effect of MutY on lowering A · T→G · C transversions in a *mutT* background has been described previously (6, 26), as has a 4.6-fold decrease in A · T→G · C mutations at one site in *trpA* (6).

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