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2-Aminopurine Allows Interspecies Recombination by a Reversible Inactivation of the *Escherichia coli* Mismatch Repair System

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2-Aminopurine treatment of *Escherichia coli* induces a reversible phenotype of DNA mismatch repair deficiency. This transient phenotype results in a 300-fold increase in the frequency of interspecies conjugational recombination with a *Salmonella enterica* serovar Typhimurium Hfr donor. This method can be used for the generation of biodiversity by allowing recombination between diverged genes and genomes.

Homologous genetic recombination depends stringently on the DNA sequence identity of the two parental molecules, as well as on the activity of the recombination and mismatch repair proteins (13). Homologous pairing of nonidentical DNA sequences results in mismatched heteroduplex molecules. Mismatched and unpaired bases are recognized by the methyl-directed mismatch repair system (MMRS), which prevents recombination between nonidentical DNA sequences (17, 21). This function of the MMRS plays a role in maintenance of the structural integrity of chromosomes (1, 12) and in genetic isolation among bacterial strains and species (8, 14, 20). For example, transductional recombination between two serovars (Typhimurium and Typhi) of *Salmonella enterica*, whose genomes differ by only 1 to 2% at the DNA sequence level, increases 10^2- to 10^3-fold in MMRS-deficient genetic backgrounds (23). Two *Escherichia coli* MMRS proteins, MutS and MutL, are required for this strong antirecombination activity, whereas the effect of other MMRS proteins, MutH and UvrD, is less pronounced (3).

The antirecombination activity of MMRS can be inhibited by the overproduction of mismatched DNA molecules, as a consequence of the MutS protein titration (6). Treatment with agents that produce DNA lesions recognized by the MMRS can also inhibit MMRS activity in *E. coli* (11, 15, 18). Whereas the resulting increase in mutagenesis has been well documented, the effect on recombination is unknown.

2-Aminopurine (2-AP) was used to investigate whether 2-AP-treated cells show a hyperrecombination phenotype and, if they do, whether it is a consequence of functional inactivation of the MMRS. Incorporation of the adenine analog 2-AP into newly synthesized DNA forms 2-AP–thymine base pairs and 2-AP–cytosine mispairs. These mismatches are apparently recognized by the MMRS, as suggested by the observation that inactivation of MMRS genes restores the resistance of *dam* mutants to 2-AP (*dam* mutants are killed by 2-AP because of MMRS activity [5]). Functional inactivation of the MMRS by 2-AP treatment was suggested by the finding that the spectrum of mutations induced in wild-type *E. coli* cells treated with high concentrations of 2-AP is not different from that of untreated MMRS mutants (2).

As a model system, we have used interspecies conjugational recombination between *S. enterica* serovar Typhimurium and *E. coli* strains. Hfr strains were *S. enterica* serovar Typhimurium SA965 and *E. coli* P4X *metB*$^+$. Recipient strains were AB1157 Nal^r derivatives constructed by P1-mediated transduction. Relevant genotypes of the recipient strains were *thr-34*::Tn*10*, *thr-3091*::Tn*10* Kan^r, *thr-34*::Tn*10 mutS*::ΩSm-Sp, and *thr-3091*::Tn*10* Kan^r *mutL218*::Tn*10*. All of the strains and alleles used in this study have been described previously (3). Plasmids expressing *mutS*$^+$ (pMQ341) and *mutL*$^+$ (pMQ339) are derivatives of the pACYC184 Cam^r vector (22). Conjugations were performed as previously described (3), except that 2-AP was added to Luria-Bertani medium during the exponential growth of recipient strains (3 h of incubation), as well as to Luria-Bertani plates on which conjugational crosses (1 h) were performed. Selection of Thr$^+$ recombinants took place on minimal M9 medium without 2-AP.

The frequency of recombination between *S. enterica* serovar Typhimurium Hfr and *E. coli* wild-type F$^-$ strains, whose genomic sequences diverge by about 20%, is very low (10^{-7} to 10^{-6}). Inactivation of the *mutS* or *mutL* gene in recipient cells increased the frequency of interspecies recombination about 10^3- to 10^4-fold (3, 13; Table 1). Treatment of wild-type recipient cells with 2-AP (concentrations of >25 μg/ml) increased the frequency of interspecies recombination nearly to, at 75 μg/ml, that obtained with *mutS* or *mutL* mutant recipients without 2-AP (Fig. 1). 2-AP concentrations of >75 μg/ml did not result in a further increase in interspecies recombination. 2-AP treatment did not significantly affect intraspecies recombination in wild-type and *mutS* bacteria or interspecies recombination in the *mutS* strain. The hyperrecombination phenotype of 2-AP-treated wild-type bacteria is reversible. (i) The absence of 2-AP only during the mating period (1 h) lowered the frequency of interspecies recombination, and (ii) 2 h of growth (five to six generations) in the absence of 2-AP eliminated the hyperrecombination phenotype of bacteria pregrown (for 3 h) with 2-AP (data not shown).

To test for potential selection of MMRS-deficient mutator mutants during growth with 2-AP prior to conjugation, the frequency of mutator mutants was analyzed among the se-

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TABLE 1. Identification of *E. coli* methyl-directed mismatch repair component depleted by 2-AP treatment

Conjugational cross and relevant genotype of recipient strain	Mean frequency of recombination[c] (SE)		Ratio[d]
	Without 2-AP	With 2-AP (300 μg/ml)	
Intraspecies[a]			
Wild type	1.4×10^{-1} (2.2×10^{-2})	1×10^{-1} (3.7×10^{-2})	0.7
mutS	1.3×10^{-1} (3×10^{-2})	8.8×10^{-2} (1.1×10^{-2})	0.6
mutS plasmid *mutS*$^+$	6.9×10^{-1} (6.3×10^{-1})	2.2×10^{-1} (1.4×10^{-1})	0.3
mutL	5.6×10^{-2} (2.5×10^{-2})	9.7×10^{-2} (4.3×10^{-2})	1.7
mutL plasmid *mutL*$^+$	5.2×10^{-2} (2.1×10^{-2})	1.7×10^{-2} (1×10^{-2})	0.3
Interspecies[b]			
Wild type	4.3×10^{-7} (1.6×10^{-7})	1.4×10^{-4} (5.9×10^{-5})	325
mutS	1.8×10^{-3} (6.7×10^{-4})	1.8×10^{-3} (7×10^{-4})	1
mutS plasmid *mutS*$^+$	1.8×10^{-7} (2.9×10^{-8})	5.6×10^{-5} (3.4×10^{-5})	311
mutL	9×10^{-4} (5.4×10^{-4})	2.2×10^{-4} (2.2×10^{-4})	0.2
mutL plasmid *mutL*$^+$	1.3×10^{-6} (2.3×10^{-7})	4.2×10^{-6} (7.5×10^{-7})	3.2

[a] *E. coli* Hfr × *E. coli* F$^-$.
[b] *S. enterica* serovar Typhimurium Hfr × *E. coli* F$^-$.
[c] Thr$^+$ recombinant-Hfr donor.
[d] Ratio of mean frequencies of recombination without/with 2-AP treatment.

lected interspecies recombinants by testing the frequency of rifampin-resistant mutants. Although the frequency of spontaneously occurring MMRS-deficient mutators in the laboratory cultures of *E. coli* is about 10^{-5} (7), we found 9% (19 out of 211) mutators among interspecies recombinants obtained with untreated wild-type recipients, corroborating published observations (4). This result is accounted for by second-order selection (19). When 2-AP (300 μg/ml)-treated wild-type cells were used as recipients, mutators represented only about 0.5% (1 out of 192) of the interspecies recombinants, showing decreased second-order selection of MMRS mutants. Therefore, it can be concluded that 2-AP induces interspecies recombination in wild-type cells.

To determine whether a specific MMRS protein is inactivated by 2-AP treatment, conjugational crosses were performed with recipient strains carrying plasmids with active *mutS*$^+$ and *mutL*$^+$ genes (Table 1). Overproduction of MutS had no effect, whereas MutL overproduction nearly completely abolished the 2-AP effect (Table 1). It is not clear why MutS is not limiting for antirecombination activity upon 2-AP treatment, as was observed with mismatched retron DNA (6). However, MutL (and not MutS) protein saturation has been observed in an *E. coli mutD5* mutator mutant that generates excessive DNA replication errors by a defect in the DnaQ proofreading subunit of DNA polymerase III (16), as well as by the base analog deoxyribosyl-dihydropyrimido[4,5-c][1, 2]oxazin-7-one (11). All of these studies on alleviation of the saturation of mismatch repair by MutS and MutL overproduction used very similar means of overproduction; i.e., increased levels of Mut proteins were achieved by increasing the copy number of the plasmid-borne natural *mutS* and *mutL* genes (in our case, about 15 copies per cell).

In conclusion, genetic barriers among enterobacteria can be alleviated in a reversible way by 2-AP treatment. Other mismatch-generating treatments may show a similar effect. Mergeay and Gerits (10) observed a transient *N*-methyl-*N*′-nitro-*N*-nitrosoguanidine-induced hyperrecombination phenotype during *E. coli* × *S. enterica* serovar Typhimurium transductional recombination well before the role of MMRS was discovered. Alleviation of genetic barriers may allow efficient

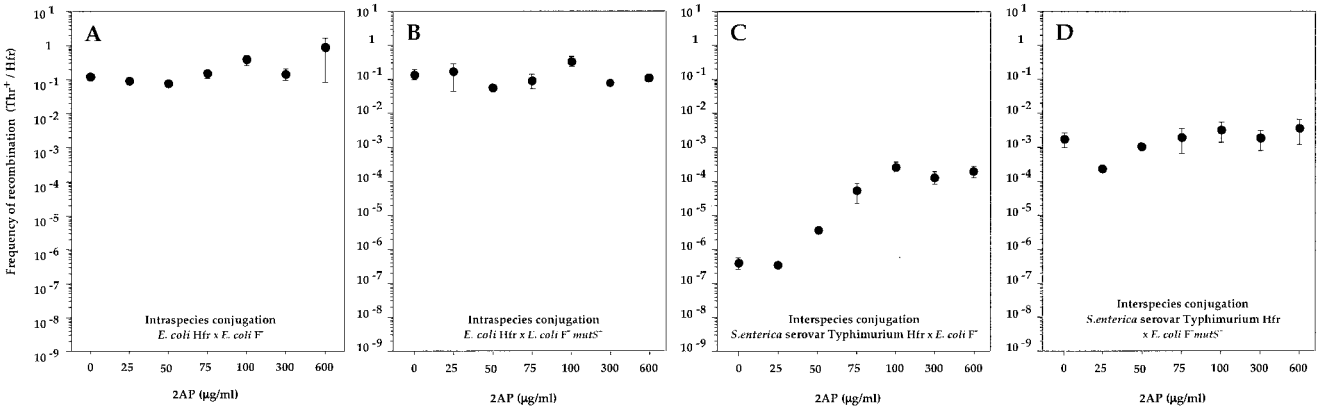

FIG. 1. Effect of 2-AP on intraspecies and interspecies conjugational recombination. Shown are the results of *E. coli* Hfr × *E. coli* F$^-$ (A), *E. coli* Hfr × *E. coli mutS* F$^-$ (B), *S. enterica* serovar Typhimurium Hfr × *E. coli* F$^-$ (C), and *S. enterica* serovar Typhimurium Hfr × *E. coli mutS* F$^-$ (D) crosses. Each value is the mean frequency of recombination obtained in at least three independent experiments (± the standard error).

interspecies recombination of genes, operons, or genomes, producing large-scale biodiversity as a useful source of biotechnological innovation (9). The advantages of this "chemical" method over the use of MMRS-deficient mutants are that (i) the genetically unstable state can be limited to the time of the interspecies cross and (ii) the procedure can be applied to different bacterial species and probably also to eukaryotic cells.

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