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Inactivation of Mismatch Repair Overcomes the Barrier to Transduction between *Salmonella typhimurium* and *Salmonella typhi*

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P22 transduction of chromosomal genes from *Salmonella typhimurium* into *Salmonella typhi* occurs at a low frequency. Transduction of plasmids from *S. typhimurium* into *S. typhi* occurs at a frequency similar to that between *S. typhimurium* strains, indicating that the barrier to transduction of chromosomal genes is not due to an inability of P22 to inject DNA into *S. typhi* or a restriction endonuclease that rapidly degrades foreign DNA. Furthermore, transduction of *mutS* and *mutL* derivatives of *S. typhi* with chromosomal genes from *S. typhimurium* occurs efficiently. These results indicate that the transduction barrier is due to activity of the recipient mismatch repair system, which senses sequence divergence and disrupts heteroduplexes in favor of recipient sequences. Inactivation of the mismatch repair system allows P22 transduction to be used as an effective tool for constructing *S. typhi-S. typhimurium* hybrids.

Salmonella typhi is a gram-negative pathogen responsible for typhoid fever, a systemic disease restricted to humans. Relatively little is known about the pathogenic mechanisms of *S. typhi* because of the lack of an adequate animal model and the lack of useful genetic tools. Consequently, *Salmonella typhimurium* has been frequently used to study aspects of typhoid fever, since it causes a typhoid-like disease in mice and has a well-developed genetic system (13). The most powerful tool of *S. typhimurium* genetics is P22, a temperate bacteriophage used to mediate generalized transduction (7–9). P22 has also been used to transduce DNA from *S. typhimurium* into *S. typhi* (5). However, P22 is not an effective tool for construction of *S. typhi-S. typhimurium* hybrids because the frequency of recombination between *S. typhi* and *S. typhimurium* is very poor. *S. typhi* can be efficiently infected by certain lytic phage grown in *S. typhimurium*, such as the lytic phage Felix-O (3, 10a), suggesting that the low frequency of transduction is not due to restriction of the foreign DNA. Alternatively, the low frequency of transduction could be due to a recombination barrier caused by slightly divergent DNA sequences in these two closely related species. For example, Rayssiguier et al. (12) showed that repair of *Salmonella* mutants by transductional recombination with *Escherichia coli* donors is severely limited because of slight differences between the two recombining sequences. Inactivation of the methyl-directed mismatch repair system encoded by *mutHSL* relaxes the requirement for DNA sequence homology and allows increased recombination between DNA from *E. coli* and *S. typhimurium* (12). MutHSL proteins interact with heteroduplex DNA such that recognition of a nucleotide mismatch promotes cleavage of the donor strand (1, 10) (Fig. 1). We report here that inactivation of the methyl-directed mismatch repair system in *S. typhi* allows efficient recombination with DNA from *S. typhimurium*.

S. typhimurium and *S. typhi* strains used in this study are shown in Table 1. Transductions were done with two derivatives of bacteriophage P22. P22 HT105/1 *int-201* contains

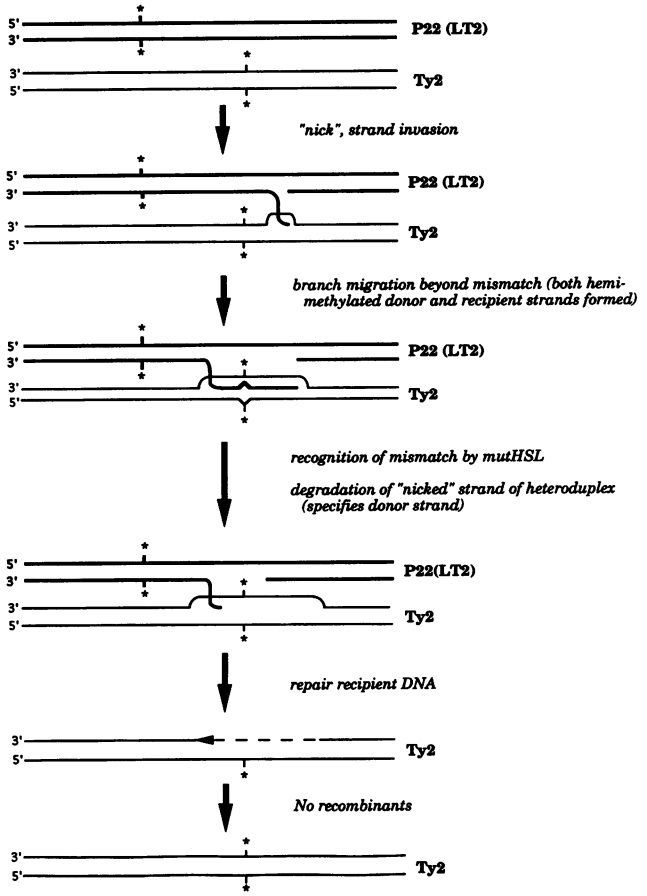

FIG. 1. Model for heteroduplex repair of transducing particles by mismatch repair. Asterisks indicate *dam* methylation sites, bold lines indicate donor DNA, and thin lines indicate recipient DNA. After strand invasion and branch migration, mismatches will occur in the resulting heteroduplex. DNA packaged in generalized transducing particles is not expected to be undermethylated, but the donor DNA and recipient DNA will be unmethylated at some positions because of polymorphisms in *dam* methylation sites. Preferential repair of the recipient DNA would occur if the MutHSL complex specifically recognizes the free end of the donor strand and cleaves the nicked strand of the heteroduplex.

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TABLE 1. Bacterial strains used in this study

Bacterial strain[a,b]	Genotype[c]	Reference or source
S. typhimurium		
LT2	Wild type	J. Roth
TT9589	*recA1 del(his HB)22*/pBR322	J. Roth
TT15222	*thr-458*::Mud*Q*(A)	2
TT15225	*pyrA2413*::Mud*P*(B)	2
TT15238	*nadA219*::Mud*P*(A)	2
TT15256	*purG2149*::Mud*P*(A)	2
MST1978	*ser-1701*::Mud*J*	Laboratory collection
MST1988	*thr-1361*::Mud*J*	Laboratory collection
MST2433	*phe-1101*::Mud*J*	Laboratory collection
MST2456	*leu-3231*::Mud*J*	Laboratory collection
MST1933	*galE496 metA22 metE55 rpsL120 xyl-404 (fels2)*$^-$ *HI-6 nml H2 enx hsdL-6 hsdSA29 mutS121*::Tn*10*	11
MST1934	galE496 metA22 metE55 rpsL120 xyl-404 *(fels2)*$^-$ *HI-6 nml H2 enx hsdL6 hsdSA29 mutL111*::Tn*10*	11
S. typhi		
Ty2	Wild type	G. Mora
TYT1002	*ser-1702*::Mud*J*	This study
TYT1132	*ser-1702*::Mud*J mutS121*::Tn*10*	This study
TYT1028	*leu-3232*::Mud*J*	This study
TYT1133	*leu-3232*::Mud*J mutS121*::Tn*10*	This study
TYT1038	*thr-1362*::Mud*J*	This study
TYT1119	*thr-1362*::Mud*J mutS121*::Tn*10*	This study
TYT1040	*phe-1102*::Mud*J*	This study
TYT1129	*phe-1102*::Mud*J mutL111*::Tn*10*	This study

[a] All strains were derived from *S. typhi* Ty2 or from *S. typhimurium* LT2.

[b] Strains were routinely grown in rich media (0.8% Difco nutrient broth with 0.5% NaCl). When indicated, ampicillin (60 μg/ml) or tetracycline hydrochloride (20 μg/ml) was added to rich media.

[c] (A) and (B) refer to the orientation of the P22 *pac* site relative to the orientation of the Mu ends (2, 16). Mud*J* is an abbreviation for Mud*I*1734, a Kan^r mini-Mu derivative that forms stable *lac* operon fusions (4). Mutants of *S. typhi* with Mud*J* insertion mutations were isolated as described by Hughes and Roth (6). Methyl-directed mismatch repair mutants of *S. typhi* were isolated by transduction of *S. typhi* with P22HT lysates grown on *mutS121*::Tn*10* or *mutL111*::Tn*10* mutants of *S. typhimurium*, with selection for tetracycline resistance. These transductions were done at a high multiplicity of infection (approximately 10 PFU per cell), because the frequency of transduction was very low.

mutations that increase the frequency of generalized transduction and prevent the formation of stable lysogens (14). Mud-P22 derivatives are phage Mu-P22 hybrids which are unable to be excised from the host genome (2, 16). Induction of this prophage with mitomycin C amplifies adjacent bacterial DNA and packages 3 to 5 min of host DNA on one side of the Mud-P22 insertion. Thus, in each Mud-P22 lysate, particular regions of the host chromosome are selectively targeted for replication and packaging into general transducing particles.

Mud-P22 lysates carry a high percentage of general transducing particles. Thus, Mud-P22 lysates allow very efficient transduction of linked genes in *S. typhimurium* (Table 2). Even with these specialized transducing lysates from *S. typhimurium*, transduction of *S. typhi* auxotrophs to prototrophy occurred at a very low frequency (Table 2). However, P22 transduction of plasmid pBR322 from *S. typhimurium* into *S. typhi* occurred at a high frequency (Table 2), indicating that the low frequency of transduction of chromosomal DNA was not merely due to the presence of a restriction modification system or the inability of P22 to infect *S. typhi*. Inheritance of plasmid pBR322 simply requires recircularization via homologous recombination within a linear concatemer, not formation of an *S. typhi-S. typhimurium* heteroduplex. These results suggested that the low frequency of transduction is due to a recombination barrier between these two species, possibly caused by differences in homologous DNA sequences. Therefore, derivatives of *S. typhi* auxotrophs with mutations in the mismatch repair system were tested for increased frequency of transduction with P22 grown on *S. typhimurium*. These auxotrophic *S. typhi mutL* or *mutS* strains were transduced to prototrophy with Mud-P22 at a much higher frequency than the corresponding *mutL*$^+$ or *mutS*$^+$ strains were (Table 2). Thus, inactivation of the methyl-directed mismatch repair system in the recipient allows efficient recombination between *S. typhi* and *S. typhimurium*.

Similar results were obtained with P22HT *int* transducing lysates (Table 2). *S. typhi* strains carrying a *mutS* or *mutL* mutation show much higher frequencies of transduction than do the corresponding *mutL*$^+$ or *mutS*$^+$ strains. Although the transduction frequency in the *S. typhi* mismatch repair mutants is lower than the frequency of transduction in *S. typhimurium*, this increase is sufficient to allow efficient transduction of *S. typhi* with the heterologous DNA.

Generalized transduction is a powerful genetic tool. Phage P22 is an indispensable tool in *S. typhimurium* genetics, but its use in *S. typhi* has been very limited. Inactivation of the methyl-directed mismatch repair system encoded by *mutSL* relaxes the requirement for DNA sequence homology and allows the efficient and effective use of P22 as a genetic tool in *S. typhi*. *S. typhi-S. typhimurium* hybrids produced by P22 transduction will be useful for genetic characterization of the *S. typhi* genome, allowing rapid identification of genetic functions that are unique or shared between these closely related bacterial pathogens.

It seems unlikely that the inhibition of recombination by the mismatch repair system is restricted to *E. coli* and *Salmonella* spp. Inactivation of the mismatch repair system of a recipient may be a general approach to facilitate efficient recombination between any two closely related species of bacteria.

TABLE 2. Frequency of transduction

Recipient strain	Genotype	Frequency of transduction of donor phage (10^7)[a]		
		P22(pBR322)	Mud-P22	P22HT *int*
S. typhi				
TYT1038	*thr*::MudJ	160	<0.01	<0.01
TYT1040	*phe*::MudJ	88	<0.01	<0.01
TYT1002	*ser*::MudJ	340	<0.01	<0.01
TYT1028	*leu*::MudJ	550	<0.01	<0.01
S. typhi mutSL				
TYT1119	*thr*::MudJ	100	3.3	0.13
TYT1129	*phe*::MudJ	360	68	3.6
TYT1132	*ser*::MudJ	210	<0.01	0.79
TYT1133	*leu*::MudJ	460	15	4.8
S. typhimurium				
TYT1988	*thr*::MudJ	200	5.3	220
TYT2433	*phe*::MudJ	2,200	81	360
TYT1978	*ser*::MudJ	200	15	1,500
TYT2456	*leu*::MudJ	130	34	1,500

[a] Recombination frequency expressed as number of transductants per milliliter of phage lysate per viable cell. Transduction frequencies have been multiplied by 10^7 (for example, <0.01 indicates $<10^{-9}$). Transductions were performed in triplicate with similar results. Controls containing only bacteria or phage did not produce prototrophic colonies. P22HT lysates were prepared as described by Maloy (7). Mud-P22 lysates were prepared as described elsewhere (2, 16). Transductions were done at a multiplicity of infection of 0.5. Transduction of pBR322 was done by selecting for growth on rich media containing ampicillin. Transduction to prototrophy was done by selecting for growth on E medium (15) containing 0.2% glucose. The Mud-P22 lysates of *S. typhimurium* used to transduce MudJ auxotrophs to prototrophy were TT15222 (*leu*$^+$), TT15225 (*thr*$^+$), TT15238 (*ser*$^+$), and TT15256 (*phe*$^+$). To avoid transfer of nutrients from the rich medium, the recipient cells were washed and resuspended in 0.85% NaCl before plating. Since *S. typhi* Ty2 is a natural cysteine-and-tryptophan auxotroph, Ty2 was supplemented with 1.05% cysteine and 0.41% tryptophan when grown on minimal media. Addition of cysteine and tryptophan did not affect the frequency of transduction of *S. typhimurium*.

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