Transduction of *Escherichia coli* *trp* Genes in *Salmonella typhimurium* and Effect of *N*-Methyl-*N'*-Nitro-*N*-Nitrosoguanidine on Transduction with Heterogenetic DNA

By MAX MERGEAY* AND JOZEF GERITS

Department of Radiobiology, SCK/CEN, Boeretang 200, B-2400 Mol, Belgium

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P1Kc-mediated transduction of *Escherichia coli* *trp* genes occurred at a frequency of about 10^{-8} in *Salmonella typhimurium* *trp* strains carrying mutations determining sensitivity to P1 and a low level of restriction enzymes. Heterospecific transductants were analysed by using them as donors in second-stage transductions mediated by bacteriophage KB *int*. One class of heterospecific transductants had the phenotype Trp⁺ Pro⁻ but were extremely unstable and reverted at high frequency (up to 80%) to the parental phenotype. The Trp⁺ Pro⁻ phenotype probably represents insertions of the *E. coli* *trp* genes in the *S. typhimurium* *pro* genes. It was stable in a RecA background.

Haploid P1-mediated heterogenotes exhibited various degrees of homology with the *trp* region of *S. typhimurium* in KB-mediated second-stage transductions: one heterogenote (MA427), which transduced the linked markers *trp*, *cysB* and *pyrF* very poorly, was used to look at the effects of the mutagen *N*-methyl-*N'*-nitro-*N*-nitrosoguanidine (MNNG) in stimulating heterologous recombination and to derive a method for positive selection of linked mutations. Following treatment with MNNG, heterospecific Trp⁺ transductants were obtained with MA427 as donor. Their yield was maximal immediately after MNNG treatment but decreased and even disappeared when transductions were carried out a few hours later. This transient process is thought to represent MNNG-induced conversion of abortive transductants to complete transductants. On the other hand, phenotypic analysis of MNNG-induced Trp⁺ heterologous transductants revealed the presence of mutations of various phenotypes. In particular, the antimutator phenotype was recognized in 15 clones among 110 MNNG-induced recombinants tested. However, most of these mutations seemed not to influence heterologous recombination.

INTRODUCTION

There were many early reports of intergeneric gene transfer in bacteria (Jones & Sneath, 1970), especially between species of *Enterobacteriaceae* (Luria & Burrows, 1957). In this family, gene transfer was mainly studied in conjugational crosses between *Escherichia coli* and *Salmonella typhimurium* (Baron *et al.*, 1959, 1968; Zinder, 1960; for a review, see Middleton & Mojica-A, 1971). Two major topics were examined in heterospecific gene transfer: gene complementation by exogenous genetic material, and integration by recombination of foreign material in the receptor genome. The possibilities of gene complementation are now considerably extended in Gram-negative bacteria, thanks to ubiquitous plasmids belonging to the incompatibility group P1 (IncP1) and to their mobilizing derivatives such as R68.44 (Hedges *et al.*, 1977), R68.45 (Johnston *et al.*, 1978), RP4::Mu cts62 (Murooka *et al.*, 1981) and RP4::Mini Mu (Van Gijsegem & Toussaint, 1982). With such tools, heterospecific gene complementation is now possible between bacteria belonging to various genera.

Abbreviations: IGP, indole glycerol phosphate; MMS, methyl methanesulphonate; MNNG, *N*-methyl-*N'*-nitro-*N*-nitrosoguanidine.

Heterospecific gene recombination depended strictly on extended homology between donor and receptor material. For example, complementation between *S. typhimurium* and *E. coli* markers carried on F-prime plasmids has been observed for numerous loci, including those for flagella synthesis and chemotaxis (De Franco *et al.*, 1979) but, in most experiments, recombination was not reported to occur: the *S. typhimurium* merodiploids carrying *E. coli* F-prime plasmids are thus stable. In those carrying F-*trp* plasmids, recombination occurred at a frequency of 10^{-8} or less (Yanofsky *et al.*, 1977). Where heterospecific recombination did occur, a significant proportion of the recombinants appear to have resulted from illegitimate or additive recombination, as shown by Mojica-A & Middleton (1972) who analysed recombinants from *E. coli* Hfr $\times$ F⁻ *S. typhimurium* crosses by P22-mediated transduction.

The *E. coli*/*S. typhimurium* system could serve as a model to study the processing of foreign genetic material, mainly by facilitating the detection and the further study of homologous and additive (illegitimate) recombination events. Furthermore, this system is suitable to see how to increase the integration of foreign genetic material in the receptor genome. For this purpose, we wanted to define the sequence of events occurring in heterospecific recombination, to by-pass possible interference due to restriction mechanisms and to the transposable elements contained in the F-plasmid used up to now in crosses between *E. coli* and *S. typhimurium*. Therefore, we chose transduction of *trp* genes from *E. coli* to *S. typhimurium*. Bacteriophage P1 was chosen to mediate transduction in *S. typhimurium* strains which carry *galE*, *hsdL* and *hsdS* mutations and are thus sensitive to P1 and deficient in two separate restriction mechanisms (Ornellas & Stocker, 1974). P1-mediated heterogenotes were found and were subjected to further genetic analysis by using bacteriophage KB *int* (which effects generalized transduction in a way very similar to P22 and is easier to manipulate in *S. typhimurium* than P1). KB lysates were grown on P1-mediated heterogenotes and used as transductional donors for further crosses with suitable *S. typhimurium* recipients. Since, in some cases, lack of homology drastically hindered recombination between heterogenotic DNA and *S. typhimurium* DNA in the *trp* region, mutagenesis with MNNG was used to stimulate heterologous recombination.

METHODS

Strains. Table 1 lists the strains of *Salmonella typhimurium* LT2 and *Escherichia coli* K12 used in this work and shows how they were obtained.

Media. Minimal medium was that of Vogel & Bonner (1956), provided with a carbon source at a concentration of 0.2% and other additions as follows: amino acids and bases $30 \mu\text{g ml}^{-1}$ and $10 \mu\text{g ml}^{-1}$, respectively; anthranilate, 2 or $10 \mu\text{g ml}^{-1}$. Selection of *trp*⁺ recombinants was usually carried out on Vogel-Bonner plates supplemented with casein hydrolysate (0.1%). Rich media included Luria broth (L broth) agar or soft agar supplemented with CaCl_2 (10^{-3} M) and DL-cysteine ($30 \mu\text{g ml}^{-1}$). These were also used for the propagation of P1 phage. For P22 or KB phage procedures, tryptone broth agar and soft agar were used (Roth, 1970).

Accumulation tests. Overnight cultures of the mutants to be tested were grown in minimal medium supplemented with limiting amounts of tryptophan ($0.5 \mu\text{g ml}^{-1}$). Samples (1 ml) were taken and mixed with the following reagents. (a) *IGP reagent*. This was a solution containing 1 ml 0.5 M- FeCl_3 , 30 ml concentrated H_2SO_4 and 50 ml H_2O . A mixture of 1 ml reagent and 1 ml culture developed a pink colour in 60 s if IGP had accumulated. (b) *Anthranilate and indole reagent*. Paradimethylbenzoic acid (9 g) was dissolved in 200 ml ethanol, 45 ml concentrated HCl was added and the mixture was brought to 250 ml with ethanol. Two drops of the reagent were added to 1 ml culture. A deep yellow reaction indicated accumulation of anthranilate. If 0.5 ml of reagent was added, a deep red reaction indicated accumulation of indole.

Bacteriophage procedures. (a) *P1 lysates*. Phage P1 was propagated on lawns made with bacteria grown up to mid-exponential phase. Phage was diluted in order to get confluent lysis, and then mixed with bacteria and L broth soft agar. L broth agar plates overlaid with such mixtures were incubated at 37 °C overnight, flooded with 4 ml L broth and then stored overnight at 4 °C. The liquid was harvested, centrifuged to remove bacterial debris, and exposed to one drop of chloroform; the resulting phage suspensions were titrated on indicator strains (usually *Shigella* 16).

Salmonella typhimurium (provided with *galE*, *hsdSA* and *hsdL* mutations) was devised by Horn and Yanofsky (personal communication). It required a high multiplicity, of 5, 10 or even 20. (Multiplicities of this order are only suitable for P1Kc, since P1vir and P1C1 can only be used at low multiplicities if excessive killing is to be avoided.) Suitable lysates of P1Kc grown on *E. coli* (5×10^{10} p.f.u. ml^{-1}) were therefore mixed with exponential cultures of *Salmonella* recipients (5×10^8 ml^{-1}). The mixture was centrifuged and concentrated 10-fold in the same buffer.

Table 1. *Bacterial strains*

Strain	Relevant genotype	Origin or source
<i>E. coli</i>		
W3110	<i>trp</i> ⁺	C. Yanofsky
$\Delta[trpA229-tonB]$	$\Delta[trpA229-tonB]$	C. Yanofsky
<i>trpE10220</i>	<i>trpE10220</i>	C. Yanofsky
<i>S. typhimurium</i>		
SL1657	<i>trpD2 metA22 metE331 ilv-452 galE496 hsdL6 hsdS29 strA120 xyl-404</i>	B. A. D. Stocker
<i>cysB403</i>	<i>cysB403</i>	C. Yanofsky
MA428	<i>cysB403 metA22 metE331 ilv-452 galE496 hsdL6 hsdS29 strA120 xyl-404</i>	Trp ⁺ Cys ⁻ transductant from SL1657 $\times$ <i>cysB403</i>
$\Delta trpBA196$	$\Delta trpBA196$	C. Yanofsky
MA421	$\Delta trpBA196 metA22 metE331 ilv-452 galE496 hsdL6 hsdS29 strA120 xyl-404$	Cys ⁺ Trp ⁻ transductant from SL1657 $\times$ <i>trpBA196</i>
<i>trpB119</i>	<i>trpB119</i>	E. Balbinder
MA413	<i>trpB119 metA22 metE331 ilv-452 galE496 hsdL6 strA120 xyl-404 hsdS29</i>	Cys ⁺ Trp ⁻ transductant from SL1657 $\times$ <i>trpB119</i>
MA451	<i>leu500 azi-451</i> + markers of MA421	Azi ^R Leu ⁻ transductant in MA421
TT215	<i>lysA565::Tn10</i>	J. R. Roth
MA411	<i>cysB529 leu-500 trpBA196</i>	Faelen <i>et al.</i> (1981)
MA594	<i>cysB529 leu-500 trpBA196 lysA565::Tn10</i>	MA411 $\times$ TT215
MA412	$\Delta pyrF140 leu-500 trpBA196$	Faelen <i>et al.</i> (1981)
MA530	$\Delta pyrF140 leu-500 trpBA196 lysA565::Tn10$	MA412 $\times$ TT215
<i>trpE49</i>	<i>trpE49</i>	E. Balbinder
MA751	<i>trpE49 lysA565::Tn10</i>	From transduction <i>trpE49</i> $\times$ TT215
<i>trpA33</i>	<i>trpA33</i>	E. Balbinder
MA802	<i>cysB529 trpA33 leu-500 lysA565::Tn10</i>	From transduction MA594 $\times$ <i>trpA33</i>
<i>trpA24</i>	<i>trpA24</i>	E. Balbinder
MA803	<i>cysB529 trpA24 leu-500 lysA565::Tn10</i>	From transduction MA594 $\times$ <i>trpA24</i>
TT521	<i>srl-202::Tn10 recA1 rpsL</i>	J. R. Roth
MA425	<i>leu-500 $\Delta[supX-trpEDCBA]$</i>	C. Yanofsky
Transductional hybrids*		
ML8	<i>'trp⁺' leu-500 azi-451 metA22 metE331 ilv-452 galE496 hsdL6 hsdS29 strA120 xyl-404</i>	See Table 4
MA427	<i>'trp⁺'</i> + markers as ML8	See Table 4
MA746	<i>'trp⁺'</i> + markers as ML8	See Table 4
MA753	<i>'trpB⁺' $\Delta[trpA229-tonB]$ metA22 metE331 ilv-452 galE496 hspL6 hspS29 strA120 xyl-404</i>	See Table 4
MA742	As MA753	See Table 4
MA743	As MA753	See Table 4
OL4	As MA753 + <i>leu-500</i> , but does not accumulate IGP	
MA748	<i>'trpB⁺'</i> + markers as MA753	See Table 4
HY5	<i>'trpB⁺' trpB119</i> + markers as MA753	See Table 4
HY8	<i>'trpB⁺'</i> + markers as MA753	See Table 4
MA432	<i>'trpE10220 trpB⁺ trpA⁺'</i> + markers as MA753	See Table 4
MA752	<i>'trpE10220' trpBA196</i> + markers as MA753	See Table 4

* *E. coli trp* markers are in inverted commas.

After incubation for 15 min, sodium citrate was added to stop further phage adsorption. Cells were then centrifuged, resuspended in minimal medium and plated (Roth, 1970).

(c) *KB int* lysates and transduction. Phage *KB int* (a gift from Dr B. A. D. Stocker) is integration-negative and transduces smooth and rough strains of *S. typhimurium* equally well (McIntire, 1974). Lysates were made by mixing 10⁶ bacteriophage and two drops of an overnight culture of a *Salmonella* strain in soft agar and pouring on tryptone agar plates. Plates were incubated at 37 °C overnight to get confluent lysis, flooded with 4 ml L broth and stored overnight at 4 °C. Harvested phage suspensions were centrifuged, titrated and stored with a few drops of chloroform. The concentrations ranged from 10¹¹ to 5 $\times$ 10¹² p.f.u. ml⁻¹. Procedures using P22 phages were identical. P22L4 and P22HT105 were the P22 derivatives most used.

(d) *Transduction with KB or P22*. One drop of overnight culture was mixed with one drop of phage suspension at a multiplicity of 0.1 to 5, directly on selective media (Ely *et al.*, 1974). Plates were incubated overnight on selective enriched media (i.e. containing Casamino acids) or for 2 d on minimal plates.

Mutagenesis with MNNG. Overnight cultures of strains to be mutagenized (MA411 or MA412) were diluted in fresh L broth and incubated again with shaking until mid-exponential phase. Cells were then washed in Tris/maleate buffer, concentrated twofold and exposed to the mutagen (200 µg ml⁻¹) for 10 min without shaking (Adelberg *et al.*, 1965). The cells were then harvested by centrifugation, washed, resuspended in L broth and exposed to transducing phage at times varying from 20 min to 24 h after MNNG treatment.

Phenotypic analysis of recombinants. Any Trp⁺ recombinants obtained after exposure of recipient cells to MNNG were checked for the presence of auxotrophic or thermosensitive mutations, and screened for mutations affecting DNA metabolism. We used the following phenotypic traits to screen recombinants: (i) increased sensitivity to UV (UV^s), nalidixic acid, 2-aminopurine or MMS; (ii) mutator activity; and (iii) antimutator activity. The latter properties were determined by scoring rates of production of spontaneous mutants which were either leucine independent or resistant to kanamycin, rifampicin or sodium selenite (Fimmel & Loughlin, 1977).

RESULTS

Heterospecific crosses between E. coli and S. typhimurium with phage P1 as vector

The *S. typhimurium* recipient strains used had mutations in the *hsdS* and *hsdL* genes, thereby impairing two restriction pathways, and also in the *galE* gene, thereby making them sensitive to bacteriophage P1 (Ornellas & Stocker, 1974). P1 transduced *E. coli trp* genes into these strains at a frequency of about 10⁻⁷–5 × 10⁻⁹, i.e. about 100-fold less than is usually observed for homologous transduction in either species. The biosynthetic pathway for tryptophan is summarized in Fig. 1. The various *trp* mutations involved in the crosses, and their respective map positions, are shown in Fig. 2; Table 2 shows their phenotypic response towards intermediary metabolites of tryptophan biosynthesis. The *trp* mutations in the recipients were always deletions, and selection of recombinants was normally made on enriched media (i.e. supplemented with Casamino acids, free of tryptophan). Crosses were made either to force recombination between different *trp* mutations in the donor and recipient, or to allow the expression of donor alleles. We therefore used recipient deletions affecting *trpB* (growth response positive only with tryptophan) and selective media containing indole [which allowed expression of the donor alleles *trpE* or *trpA* (Table 2)].

The results of such transductions and the main phenotypic classes observed among the viable recombinants are shown in Table 3. In some transductions, we observed a large proportion of flat translucent colonies (20–50 times more frequent than the large transductant colonies). The nature of these colonies was not investigated; they may represent abortive transductants.

The crosses in table 3 describe four possible situations: transduction of wild-type *E. coli trp* genes (cross 1); transduction between deletions affecting *trpA* (cross 2); transduction between complementing mutations lying in adjacent genes (*trpB* and *trpA*) (cross 3); and transduction between complementing mutations lying in non-adjacent genes (*trpE* and *trpB*) (cross 4). (The main difference between transductional crosses 3 and 4 is the distance allowed for possible crossovers in the interval.) The phenotypic classes recovered from each cross are shown in Table 3. In cross 2, Trp⁺ recombinants were never found, either by direct selection (minimal medium plus Casamino acids), or by supplementing minimal medium plus Casamino acids with indole and 5-methyltryptophan. The latter selection is the phenotypic tool used to recover possible CRM⁺ (cross-reacting material) recombinants with a Trp⁻ phenotype. Protein extracts of TrpA⁻ CRM⁺ (or missense) mutants would react with antibodies prepared against the tryptophan synthetase α subunit specified by the *trpA* gene. All transductants carried the donor allele Δ[*trpA229-tonB*]. Among 1000 clones analysed, one recombinant exhibited a requirement either for a mixture of serine and indole, or for tryptophan. Crosses 3 and 4 between complementing mutations gave rise to Trp⁺ recombinants; they were the majority in cross 3 (Table 3). Approximately half of the Trp⁺ recombinants obtained in cross 3 were unstable and had further auxotrophic requirements; among the unstable recombinants about half required proline and the rest would grow only in the presence of Casamino acids. If selection was not maintained, these recombinants rapidly lost their additional auxotrophy and could then be

Fig. 1. Schematized biosynthetic pathway for tryptophan (from Crawford, 1975). Metabolic steps are shown with their corresponding genes in *E. coli* and *S. typhimurium*.

Fig. 2. Map position of relevant markers from the *pyrF-tonB* region in *E. coli* and *S. typhimurium*.

Table 2. Phenotype of <i>trp</i> mutations				
Allele* . . .	<i>trpE10220</i>	$\Delta[trpA229-tonB]$	<i>trpB119</i>	$\Delta trpBA196$
Growth response				
Minimal medium	—	—	—	—
+ Anthranilic acid	+	—	—	—
+ Indole	+	+	—	—
+ Indole and 5-methyltryptophan	+	—	—	—
+ Tryptophan	+	+	+	+
Accumulation test				
Anthranilic acid	—	—	—	—
IGP	—	+	+	+
Indole	—	—	—	—

* See Fig. 2 for origin and map position.

Table 3. *P1-mediated interspecific transductional crosses*

The recipients were *S. typhimurium* strains MA421 (crosses 1, 2, 4) and MA413 (cross 3). P1Kc donor lysates were grown on *E. coli* strains W3110, $\Delta[trpA229-tonB]$ or *trpE10220* (Table 1). In every cross, TrpB⁺ recombinants were selected.

Cross	Recipient allele	Donor alleles	No. of recombinants analysed	Phenotypic classes*
1	$\Delta trpBA196$	<i>trp</i> ⁺	25	Trp ⁺ (25)
2	$\Delta trpBA196$	$\Delta[trpA229-tonB]$	1000	Ind ⁻ (999), Ind + serine ⁻ (1)
3	$\Delta trpB119$	$\Delta[trpA229-tonB]$	200	Trp ⁺ (75), Ind ⁻ (70), Trp ⁺ X ⁻ (55)
4	$\Delta trpBA196$	<i>trpE10220</i>	25	Anth ⁻ (22), Trp ⁺ X ⁻ (3)

* Anth⁻, requiring anthranilate; Ind⁻, requiring indole; Ind + serine⁻, requiring indole and serine; Trp⁺X⁻, tryptophan-independent clone with an additional auxotrophy. The numbers in parentheses are the numbers of recombinants analysed that exhibited the indicated phenotype.

shown to contain only the recipient *trp* allele. In cross 4, three recombinants were found with a similar phenotype, one requiring proline and the other two, Casamino acids. The recovery of these unstable Trp⁺ recombinants suggests the existence of a mechanism for illegitimate recombination involving insertion of donor *trp* genes outside the *trp* operon of the recipient.

KB-mediated transduction via heterogenotes

KB phage lysates were prepared on some of the heterospecific transductants from the experiments reported above and used to transduce *trp cysB* and *pyrF* mutants of *S. typhimurium* (Fig. 2). Table 4 reports results of transduction with three types of lysates grown on heterogenotes containing *trp*⁺, $\Delta[trpA229-tonB]$ and *trpE10220* *E. coli* alleles and coming from crosses 1, 2, 3 and 4 (Table 3). These transductions allowed us to estimate the homology of heterogenotic DNA relative to the parental *trp* regions. Table 4 shows, for every heterogenote, which parental markers were recovered in transduction (generally by transducing a *cysB* marker cotransducible with the *trp* region). For example, if from a Trp⁺ *S. typhimurium* heterogenote the parental markers *trpB119* or $\Delta trpBA196$ (Table 3) were recovered among *cysB*⁺ transductants, the heterogenote could be regarded as diploid for the *trp* region. This was the case for heterogenotes MA752 and HY5 and about 15% of Trp⁺ P1-mediated heterogenotes found in cross 3 (Table 3). They will be discussed below. Heterogenotes in which the parental *S. typhimurium* marker was not recovered (i.e. not found by cotransduction with *cysB* marker) are likely to have arisen by substitution of *S. typhimurium* DNA by *E. coli* DNA.

An obvious case of substitution was observed in the heterogenote MA432, where the parental $\Delta trpBA196$ marker was replaced by the *E. coli* marker *trpE10220*, probably introducing most of the *E. coli trp* operon in *S. typhimurium* (Fig. 2). MA742 and MA743 may be interpreted in a similar way. The use of strains such as MA742 or MA432 as receptors allowed us to estimate the homology between heterogenotic *trp*⁺ DNA and *E. coli* DNA (Table 4). Homology of a heterogenote relative to parental *trp* regions may be estimated by second-stage KB-mediated transductions (Mojica-A & Middleton, 1972). A decrease in transduction efficiency of a marker indicated a lack of homology and helped to define the extent of the substitution of receptor DNA by *E. coli* DNA in the heterogenote studied. The results reported in Table 4 show various patterns of homology for the haploid heterogenotes: a correlation is observed between low transduction of *S. typhimurium trp* markers by a lysate grown on a heterogenote and a high level of transduction of the corresponding *E. coli trp* marker, and vice versa.

Thus, increased homology with the *E. coli trp* region parallels a decreased homology with the DNA of *S. typhimurium trp* region. Using such correlations we interpret hybrids MA748 and HY8 (Table 4) as recombinants obtained by legitimate crossovers between the alleles *trpB119* and $\Delta[trpA229-tonB]$ (Fig. 2). Data from Table 4 show that the homology with *S. typhimurium* DNA was poor at the left of the expected cross-over (where *E. coli* DNA is expected to replace

Table 4. Transduction of *S. typhimurium* LT2 strains by *KB* lysates grown on *P1*-mediated heterogenotes

All the *P1*-mediated heterogenotes were obtained from strains MA421 and MA413 (Table 1), and acted as donors in *KB*-mediated second-stage transductions. Recipient *S. typhimurium* strains harboured the *lysA565* : : *Tn10* insertion as outside marker and a mutation in the *pyrF-trpA* region (Fig. 2). Transduction frequencies of markers of the *pyrF-trpA* region are normalized relative to the transduction frequency of the outside marker.

Hetero- gene cross*	Pheno- type	<i>trp</i> markers detected†	Transduction frequency of marker alleles:									
			<i>lysA565</i> : : <i>Tn10</i>	$\Delta pyrF140$	<i>cysB403</i>	<i>trpE49</i>	<i>trpB119</i>	<i>trpBA196</i>	<i>trpA24</i>	<i>trpA33</i>	<i>trpE10220</i> ‡	$\Delta trpA229-tonB$ §
ML8	1	Trp ⁺	100	50	10	0	0	0	0	0	70	70
MA427	1	Trp ⁺	100	0.01	0	0	0	0	0	0	60	60
MA746	1	Trp ⁺	100	74	62	5	0.33	0.45	0.4	0.4	55	60
MA753	2	Ind ⁻	100	100	100		58	80				0
MA742	3	Ind ⁻	100	110	142			219				0
MA743	3	Ind ⁻	100	94	55			0.62				0
MA748	3	Trp ⁺	100	0.53	0.13	0.5	0.45	0.13		2.7	27.6	0
HY5	3	Trp ⁺	100	120	60		0.68	8	4.5	9.3	100	0
		<i>trpB</i> ⁺ and <i>trpB119</i> (LT2)										
HY8	3	Trp ⁺	100	0	0		0.4	0.5	1.3	7.5	150	1
MA432	4	Anth ⁻	100	2.6	1.6		3.5¶	4.2			0	80¶
MA752	4	Trp ⁺	100	80	65	81		58		89	0	65
		<i>trpE10220</i> and <i>trpBA196</i> (LT2)										
Wild type (LT2)	—	Trp ⁺	100	110	110		100	100	100	100	0.01	≤0.1

* See Table 3.

† *E. coli* markers are shown as such. Markers from *S. typhimurium* are followed by LT2 in parentheses. Detection of *trp* *S. typhimurium* parental markers was done by transducing strain *cysB403* and scoring 60–200 Cys⁺ transductants for the presence of *trpB119* or *trpBA196*.

‡ From the heterogenote strain MA432.

§ From the heterogenote strain MA753.

|| Selection of recombinant made on minimal medium supplemented with indole in order to express the donor allele.

¶ Selection on minimal medium supplemented with anthranilate in order to express the donor allele.

Table 5. *Heterologous recombination between trpA alleles*

Recipient strain	Relevant genotype	Donor strain	Relevant genotype	Frequency of <i>trpA</i> ⁺ (%) [*]
MA753	$\Delta[trpA229-tonB]$	MA802	<i>trpA33 lysA565::Tn10</i>	0.15
MA753	$\Delta[trpA229-tonB]$	MA803	<i>trpA24 lysA565::Tn10</i>	0.2

^{*} *TrpA*⁺ transductants were measured relative to the transduction of tetracycline resistance carried by the Tn10 insertion.

recipient DNA) but was improved on the right side of the expected cross-over. On the other hand, the substitution of *S. typhimurium* DNA by *E. coli* DNA in the P1-mediated heterogenotes may extend as far as the *cysB* and the *pyrF* genes: this is the case in the P1-mediated heterogenotes MA748, MA427, HY8 and MA432.

MA427 is of special interest because a lysate grown on this heterogenote did not transduce any *trp* or *cysB* marker and transduced a *pyrF* marker very poorly. This mutant was therefore used for studies of the stimulation of heterologous recombination by mutagenic agents (see below). It is suggested that in recombinants such as MA427 the size of substitution exceeded the size of DNA particles encapsidated by bacteriophage KB or P22. These DNA particles would therefore not be transduced in second-stage transduction, due to a critical lack of homology. It is known that bacteriophages KB and P22 encapsidate DNA pieces less than half the size of those that can be carried by phage P1 (Mojica-A, 1975). This suggestion is supported by the fact that a P1 lysate grown on MA427 can transduce to *trp*⁺ the marker $\Delta trpBA196$ of MA421, while a KB *int* lysate cannot.

Finally we observed that lysates grown on P1-mediated heterogenotes that had inherited the deletion $\Delta[trpA229-tonB]$ (e.g. MA753 and MA742) often transduced very efficiently any *S. typhimurium trp* marker, which was easily replaced by the *E. coli trp* deletion marker. Deletions have already been shown to influence recombination and transduction frequencies (Chelala & Margolin, 1974). One could consider here that a deletion could decrease the distance between sites necessary for an efficient replacement of the recipient material by the donor one. On the other hand, using MA753 (carrying the $\Delta[trpA229-tonB]$ deletion) as recipient, and lysates grown on *S. typhimurium* strains carrying *trpA* point mutations (*trpA24*, *trpA33*), it was possible to get *TrpA*⁺ recombinants (Table 5); this confirms for the *trpA* gene the possibility of intragenic heterospecific recombinations (Yanofsky *et al.*, 1977; Schneider *et al.*, 1981).

Table 4 also shows results for the P1-mediated heterogenotes MA752 and HY5, which were found to be diploid for the *trp* region. Although phenotypically *Trp*⁺, they also carried the parental *S. typhimurium trp* marker. MA752 is a stable *Trp*⁺ derivative spontaneously obtained from the highly unstable *Trp*⁺ X⁻ P1-mediated transductants quoted in Table 3 and described below in more detail. In this heterogenote, the *E. coli trp* marker was not linked to the *cys-trp* region of *S. typhimurium* (Table 6), but it was apparently transduced like a Tn10 insertion into the various recipients (Table 4). On the other hand, in HY5, the *E. coli trpB*⁺ marker was contrasducible with the markers of the *cys-trp* region (Table 6). A KB *int* lysate grown on HY5 transduced to *trp*⁺ any *trp* markers of *S. typhimurium* at somewhat lower frequencies (Table 4): it looked like an impairing effect of heterologous DNA in a homologous recombination process, as for example with HY8 or MA746 used as donors. The P1-mediated heterogenote HY5 could thus have originated in the original P1-mediated transduction (cross 3) from a circular intermediate. In this case, legitimate recombination would give rise to recombinants partially diploid for the *trp* markers. In cross 3, about 10% of the recombinants were similar to HY5 (diploid, and foreign *trp* marker integrated in the *S. typhimurium trp* region). Recombinants sharing the same characteristics were also described in conjugational studies by Mojica-A & Middleton (1972) and Johnson *et al.* (1975). All these recombinants seemed to be analogous to tandem duplications produced in genetical crosses as reviewed by Anderson & Roth (1977). Heterology should make such recombinants more stable than normal tandem duplications. In fact, they are difficult to store for a long time, in contrast to derivatives such as MA752.

Table 6. Genetic analysis of P1-mediated heterogenotes

Transductions were made with, as donors, KB lysates grown on the Trp⁺ P1-mediated heterogenotes MA752 and HY5. Transductions with *cysB403* as recipient indicate the diploidy of MA752 and HY5 for the *trp* region. Transductions with MA411 indicate whether or not the *E. coli trp*⁺ allele for the heterogenotes is linked to *cysB*. Transductions with MA425 indicate whether the *E. coli trp*⁺ marker of MA752 is linked with *supX*. This locus, acting on the *leu-500* allele, lies between *cysB* and *trpE* (Thorne & Corwin, 1972). *supX* recombinants are Leu-500⁺. Transduction with haploid heterogenote HY8 as donor is shown as control.

Recipient	Relevant genotype	Donor	Selected marker	Unselected marker	Frequency of unselected marker
<i>cysB403</i>	<i>cysB403</i>	MA752	Cys ⁺	$\Delta trpBA196$	30/240
<i>cysB403</i>	<i>cysB403</i>	HY5	Cys ⁺	<i>trpB119</i>	15/120
MA411	<i>cysB529</i> $\Delta trpBA196$	MA752	Cys ⁺	<i>trp</i> ⁺	0/500
MA411	<i>cysB529</i> $\Delta trpBA196$	HY5	Cys ⁺	<i>trp</i> ⁺	10/240
MA411	<i>cysB529</i> $\Delta trpBA196$	MA752	Trp ⁺	<i>cys</i> ⁺	0/600
MA411	<i>cysB529</i> $\Delta trpBA196$	HY5	Trp ⁺	<i>cys</i> ⁺	12/200
MA425	<i>leu-500</i> $\Delta supXtrpEDCBA$	MA752	TrpB ⁺	<i>supX</i> ⁺	0/115
			TrpB ⁺	<i>trpE10220</i>	115/115
MA425	<i>leu-500</i> $\Delta supXtrpEDCBA$	HY5	TrpB ⁺	<i>supX</i> ⁺	no transductants found
MA425	<i>leu-500</i> $\Delta supXtrpEDCBA$	HY8	TrpB ⁺	<i>supX</i> ⁺	45/45

Detection of 'cryptic *trp* alleles'

Mojica-A & Middleton (1972) observed the production of cryptic *trp* alleles (i.e. different from either parental one) in intergeneric conjugational crosses. We made similar observations in P1-mediated heterospecific transduction in cross 2 (Table 3). In this cross, the recombinants all required indole to grow, were sensitive to 5-methyltryptophan (TrpA⁻ CRM⁻ negative phenotype) and to chromium (TonB⁻ phenotype), thus indicating a replacement of the recipient allele *trpBA196* by the *E. coli* $\Delta[trpA229-tonB]$ marker. However, among 120 P1-mediated transductants submitted to phenotypic analysis, 5 failed to accumulate IGP, in contrast to both parents (Table 2); this suggests that they may have a block 'upstream' in the biosynthetic steps governed by the genes *trpE*, *D* or *C*. This block would be a 'cryptic' *trp* allele, and should be separable from the $\Delta[trpA229-tonB]$ allele by transduction. Therefore, KB *int* lysates were prepared on these IGP non-accumulating intergeneric transductants (like OL4) (Table 2) and were used to transduce strains carrying the *trpB119* allele (strains MA413 or MA424), selection being made on media supplemented with anthranilate in order to recover cross-overs between *trpB119* and $\Delta[trpA229-tonB]$.

Of 40 recombinants obtained in the transduction MA413 $\times$ OL4, 30 required anthranilate to grow and thus had a TrpE⁻ phenotype. Similar results were obtained with the other IGP non-accumulating P1-mediated heterogenotes from cross 2. Moreover, 'cryptic alleles' so recovered differed phenotypically from one another. [In control experiments with strains MA421 and MA413 carrying $\Delta trpBA196$ and *trpB119* and transduced by a *trp*⁺ donor lysate, respectively 3000 and 1000 *trpB*⁺ transductants selected on minimal medium plus anthranilate were all prototrophic. Similarly, in a homologous transduction (MA413 $\times$ *trpA33*) designed to simulate the transduction MA413 $\times$ OL4 and to force a crossover between a *trpA* and a *trpB* allele, 200 *trpB*⁺ selected on minimal medium plus anthranilate were also found to be prototrophic.] The heterogenotes like OL4 could thus be the result of a legitimate cross-over between *E. coli* and *S. typhimurium trp* DNA occurring in the *trpE* region and leading to an impaired heterogenotic product (for example, by a conversion consecutive to one or several mismatches).

Analysis of Trp⁺ X⁻ transductants from crosses 3 and 4

These recombinants (Table 3) showed two associated characteristics: the Trp⁺ phenotype plus a new auxotrophic requirement, and an extreme instability. Among such recombinants, the new requirement was very often for proline.

Table 7. *Stability of Trp⁺ Pro⁻ clones*

A P1-mediated transductant from cross 3 (MA413 $\times$ $\Delta[trpA229-tonB]$; Table 3), exhibiting the phenotype Trp⁺ Pro⁻ and highly unstable, was transduced with a KB *int* lysate grown on TT215 in order to receive the markers *srI-202* :Tn10 and *recA*. Both *rec⁺* and *recA* derivatives were tested for stability after growth on minimal medium supplemented with Casamino acids (and thus without tryptophan). Cultures were plated on media deprived either of proline, or of proline and tryptophan. Frequencies of colonies are reported versus the viable count estimated on L-broth agar and on minimal medium supplemented with tryptophan and Casamino acids.

<i>rec</i> allele	Frequency of spontaneous subclones	
	Trp ⁻ Pro ⁺	Trp ⁺ Pro ⁺
<i>rec⁺</i>	$2-8 \times 10^{-1}$	10^{-4}
<i>recA</i>	10^{-6}	10^{-4}

Instability was reflected in a simultaneous loss of proline (or other) auxotrophy and tryptophan independence at very high frequency (up to 80%); the loss of these characters became evident when it was found that streaking subcultures on L broth agar or media containing tryptophan immediately led to the loss of both Trp⁺ and Pro⁻ phenotypes. On the other hand, if subcultured on minimal media lacking both proline and tryptophan, these recombinants produced Trp⁺ Pro⁺ subclones at a frequency of 10^{-4} . These subclones were fully stable and were found for every Trp⁺ Pro⁻ isolate obtained in our P1-mediated intergeneric transduction experiments. Genetic analysis of the original Trp⁺ Pro⁻ strains was not possible owing to their instability. With one of the stable Trp⁺ Pro⁺ derivatives (e.g. MA752, a stable subclone arising from a Trp⁺ Pro⁻ transductant) it was found that: (a) a KB lysate grown on MA752 could not transduce *trpE10220* to Trp⁺ but could efficiently transduce $\Delta[trpA229-tonB]$ (Table 4); (b) MA752 still contained the $\Delta trpBA196$ allele and was thus diploid (Table 6); (c) all *S. typhimurium* *trp* markers were efficiently transduced (Table 4); (d) the *E. coli* *trp* fragment (probably containing the *trpEDCBA* region with the marker *trpE10220*) was not linked to the *pyrF-cysB-trp* region of *S. typhimurium* (Table 6).

It thus seemed that unstable Trp⁺ Pro⁻ transductants (and their secondary stable Trp⁺ Pro⁺ derivatives) were insertions of *E. coli* *trp* material in the genome of *S. typhimurium* outside the *trp* region. To study the nature of the instability of these Trp⁺ Pro⁻ clones that gave rise either to parental types (Trp⁻ Pro⁺) at high frequency or to stable Trp⁺ Pro⁺ types at low frequency (10^{-4}), we introduced the *recA1* marker into a Trp⁺ Pro⁻ strain (obtained from cross 3) by cotransduction with *srI202* :Tn10. This transduction was made on minimal medium deprived of tryptophan but supplemented with proline and containing tetracycline. Following purification of 12 *recA* derivatives, it was found that *recA1* strongly stabilized the Trp⁺ Pro⁻ phenotype (Table 7). The Trp⁺ Pro⁻ phenotype could therefore be maintained, but even so the frequency with which secondary Trp⁺ Pro⁺ derivatives arose was not affected. One event (Trp⁺ Pro⁻ $\rightarrow$ Trp⁻ Pro⁺) was thus clearly *recA*-dependent, while the other (Trp⁺ Pro⁻ $\rightarrow$ Trp⁺ Pro⁺) seemed to be *recA*-independent.

Use of MNNG in attempts to increase heterospecific recombination

The experiments described in this section were carried out using the hybrid MA427 (Table 4) as transductional donor. KB lysates grown on this heterogenote transduced *Salmonella* *trp* and *cysB* markers at frequencies lower than 10^{-10} (Table 4). It was felt that this would allow strong positive selection for heterospecific recombinants which might be generated following mutagenic treatment of recipient cultures.

Following the rationale described by Eisenstark (1965*a, b*) and by Colson & Colson (1967), cultures of *S. typhimurium* strains (MA411 and MA412) carrying the deletion allele *trpBA196* were treated with MNNG prior to their use as recipients in transduction experiments with phage grown on MA427 as donor, selection being made for Trp⁺ recombinants. However, in initial experiments, MNNG-treated cultures of MA411 and MA412 failed to produce any Trp⁺ transductants if they were allowed to grow for up to 24 h before transduction, as is normally

Fig. 3. Effects of MNNG on the transduction of heterogenetic *E. coli* DNA in *S. typhimurium* strains MA412 (a) and MA411 (b). Recipient cultures containing the $\Delta trpBA196$ deletion were exposed to MNNG and infected by KB phage grown on the heterogenote MA427 at different times after exposure to MNNG. The arrows indicate the total time of exposure to the mutagen, including the first centrifugation. Zero time indicates the resuspension in warm broth after washing the pelleted cells in Tris/maleate buffer. For each point, cells were mixed with KB lysate grown on MA427 at a multiplicity of 2–10 and plated on minimal plates supplemented with Casamino acids and indole. For each transduction, 10–15 plates each containing about 10^9 cells were plated.

recommended to allow full expression of newly-induced mutations (Adelberg *et al.*, 1965; Miller, 1972). We therefore carried out transductions at much earlier times after exposure to mutagen, and found that this led to a recovery of substantial numbers of Trp⁺ recombinants. The production of Trp⁺ transductants was determined as a function of time after mutagen exposure for two different recipients; Trp⁺ transductants were observed immediately, but the numbers declined rapidly during the first 2 h (Fig. 3). Of 73 Trp⁺ transductants obtained with strain MA412, 3 had also inherited the linked marker *pyrF*⁺, while among 37 Trp⁺ transductants obtained with strain MA411, 6 had inherited the linked marker *cys*⁺. Genetic analysis of some of the MNNG-induced transductants (e.g. MA412-37 and MA411-29) (Table 8), using the techniques shown in Tables 4 and 6, indicated that they were haploid for the *trp* region. On the other hand, at least two other MNNG-induced transductants segregated back to the Trp⁻ phenotype. Thus, both legitimate and illegitimate recombination was detected among MNNG-induced transductants.

All 110 Trp⁺ transductants were then subjected to phenotypic analysis as described in Methods, including a search for phenotypes associated with changes in DNA metabolism *sensu lato* and especially those reported in association with 'Hyperrec' mutants of *E. coli* (Konrad, 1977). About 70% of the 110 heterospecific transductants obtained following exposure to MNNG harboured one or more recognizable mutations, including auxotrophic, temperature-sensitive, UV-sensitive, MMS-sensitive, mutator and antimutator classes. The procedure therefore appeared to act as a powerful direct selection for various classes of mutations.

Are some of these mutations able to stimulate transduction with heterogenetic DNA? The MNNG-induced Trp⁺ transductants still have a marker (*cysB* or $\Delta pyrF140$) covered by the hybrid segment of the heterogenote MA427 (Table 8). Therefore, a mutation increasing heterologous recombination should facilitate the transduction of *cysB529* or $\Delta pyrF140$ by a KB lysate grown on MA427. Table 8 reports such transduction attempts for MNNG-induced transductants obtained from the experiments shown in Fig. 3, and exhibiting a variety of phenotypes. No MNNG-induced transductant was found to be stimulated in the further transduction of *cysB* or $\Delta pyrF140$ by a lysate grown on MA427. This suggests that most of the detected mutations are not related to the stimulation of heterologous transduction. This stimulation appeared to be an inducible and transient physiological phenomenon.

A striking feature of the experiments reported in Fig. 3 was the recovery of several antimutator clones in which the frequency of spontaneous mutagenesis was decreased by a factor of 20–500 (as judged by attempts to produce mutants resistant to kanamycin, spectinomycin or sodium selenite (Kan^R, Spc^R or Sel^R), or prototrophic revertants (Leu-500⁺)

Table 8. *MNNG-induced Trp⁺ recombinants tested for increased transduction by heterogenetic DNA*

The MNNG-induced Trp⁺ transductants came either from strain MA411 or from strain MA412 (Fig. 3). Every indicated phenotype corresponded to one allele. Thermosensitivity corresponds to growth inhibition on L broth agar at 42 °C. Antimutator phenotype indicates a decrease of at least 20-fold in spontaneous production of colonies resistant to kanamycin and of Leu⁺ revertants. Mutator phenotype indicates a 40-fold increase in colonies resistant to kanamycin.

The transduction test was made with a KB lysate grown on the heterogenote MA427 (Table 4) as a donor; *cysB* and *pyrF* markers were included in the heterologous segment of MA427. None of the MNNG-induced transductants exhibited increased transduction by the heterogenetic DNA.

MNNG-induced Trp ⁺ transductant	Phenotype of comutations	Tested marker
MA411-12	Antimutator	<i>cysB</i> ⁺
MA411-13	Thermosensitive	<i>cysB</i> ⁺
MA411-28	Antimutator	<i>cysB</i> ⁺
MA411-29	Antimutator	<i>cysB</i> ⁺
	Ilv ⁻	
MA411-37	Antimutator	<i>cysB</i> ⁺
MA412-1	UV-sensitive	<i>pyrF</i> ⁺
MA412-37	Antimutator	<i>pyrF</i> ⁺
	Thermosensitive	
	His ⁻	
MA412-46	Thermosensitive	<i>pyrF</i> ⁺
	2-Aminopurine sensitive	
MA412-60	Antimutator	<i>pyrF</i> ⁺
	Ilv A	
MA412-66	Antimutator	<i>pyrF</i> ⁺
MA412-67	Wild type	<i>pyrF</i> ⁺
MA412-72	Mutator	<i>pyrF</i> ⁺

(Mergeay *et al.*, 1981). This antimutator phenotype was previously reported in *E. coli* by Geiger & Speyer (1977). These 'antimutators' were kept for further analysis, including mapping of the mutations. These data will be published elsewhere (Mergeay *et al.*, 1981 and in preparation).

DISCUSSION

The present work shows that bacteriophage P1 was able to mediate heterospecific transfer of *trp* genes from *E. coli* to *S. typhimurium* strains carrying appropriate mutations (*galE*, *hsdS29* and *hsdL6*).

P1-mediated heterospecific transductants were analysed by second-stage KB-mediated transduction by a method similar to that used by Mojica-A & Middleton (1972) to study the events observed in conjugational gene transfer from *E. coli* Hfr strains to *S. typhimurium* F⁻ strains. Analysis by second-stage transduction allowed us to discriminate between recombinants haploid for the *trp* region and illegitimate recombinants (additive, site-specific, unequal, etc.) which are diploid for the *trp* genes. Illegitimate recombinants (or more generally any recombination not due to substitution of the recipient allele by the donor one) represented a significant fraction of the heterogenotes. Most of the events (haploid or diploid for the *trp* genes) found in the present work were comparable to those classified in heterospecific conjugation by Mojica-A & Middleton (1972). In addition, a new class of illegitimate recombinants was found which displayed the phenotype Trp⁺ X⁻ and, in particular, the phenotype Trp⁺ Pro⁻. These clones were found to be extremely unstable, losing at high frequency both their Trp⁺ character and their requirement for proline. However, the Trp⁺ Pro⁻ phenotype could be stabilized by *recA*. These heterospecific Trp⁺ Pro⁻ recombinants also gave rise to stable Trp⁺ Pro⁺ subclones still containing the original recipient *trp* allele (*trpB119* or Δ *trpBA196*). Genetic analysis of these stable Trp⁺ Pro⁺ subclones showed that the *E. coli trp* region was unlinked to the *S. typhimurium trp* region and could be retransduced in *trp* strains of *S. typhimurium* just as a transposon like

Tn10 or Tn5 would be transduced, i.e. at normal transduction frequencies and apparently without interfering with the *trp* region of *S. typhimurium* (Tables 4 and 6). Thus P1-mediated heterospecific transduction of *E. coli trp* genes could lead to the formation of stable insertions lying outside the *trp* region and having some analogies with transposons. Such analogies should be elucidated by further genetic and molecular studies.

The inventory of events occurring in heterospecific recombination led us to the main question we tried to tackle: how to increase heterospecific recombination, which is severely hindered by a lack of homology between *E. coli* and *S. typhimurium* (which nevertheless share the same gene order in the *pyrF-cysB-trp-tonB* region) (Bachmann & Low, 1980; Sanderson & Hartman, 1978)? To see how this heterology barrier opposes efficient recombination, the gene *trpA* may be taken as an example. The DNA sequence of *E. coli trpA* shows 75.2% homology with that of *S. typhimurium*; the corresponding protein sequences are 85.1% similar (Nichols & Yanofsky, 1979; Crawford & Stauffer, 1980). To stimulate heterospecific recombination, we used MNNG mutagenesis, and chose as a tool the heterogenote MA427, which, as a donor in KB-mediated transductions, could not transduce *S. typhimurium trp* markers. As shown in Fig. 3 MNNG was efficient in promoting recombination in transductions with hybrid MA427 as a donor. This effect appeared to be accompanied by an enrichment among the recombinants recovered in classes of mutants with various phenotypes, some of which affect DNA metabolism. However, as shown in Fig. 3, the effect of MNNG was transient and strongly dependent upon the time elapsed between treatment and transduction. The results in Table 8 also suggest that the mutations found in this experiment did not enhance recombination with heterogenetic DNA. In theory, possible 'Hyperrec' mutations of the type being sought should have arisen only after an appropriate time for expression following mutagenesis. This was not the case for the mutants we isolated. The experiments reported in Fig. 3 suggest that promotion of interspecific recombination by MNNG was a temporary phenomenon, and might, for example, result from MNNG-induced DNA damage (and its repair) generating new sites for the integration of DNA (Hong & Ames, 1971). The mutations exhibiting the various phenotypes observed might therefore be mutations of the type often attributed to MNNG.

Two sets of observations support these suggestions. (1) Mutagens are known to stimulate recombination (for a review see Eisenstark, 1977), and more precisely to stimulate the conversion of abortive transductants into complete transductants; the latter has been reported both for UV (Benzinger & Hartman, 1962) and for nitrous acid (Abye, 1962). It is possible that a similar phenomenon occurred in the experiments reported in Fig. 3, since the maximum frequency of *trp*⁺ recombinants induced by MNNG was similar to the frequency of heterospecific P1-mediated recombination (10^{-8}) and may therefore reflect the MNNG-induced conversion of abortive transductants to complete transductants. (2) Mutations induced by MNNG have been described by several authors and they seem to occur preferentially in loci undergoing DNA replication, thereby allowing mapping of genes relative to the replication origin (Cerdà-Olmedo *et al.*, 1968; Guerola *et al.*, 1971; Altenbern, 1973; Oeschger & Berlyn, 1974). It is possible that, in order to survive MNNG treatment and give rise to a recombinant, cells must mutate as they do. Perhaps these mutations (like those exhibiting the 'antimutator' phenotype: Mergeay *et al.*, 1981) or others found during the experiments shown in Fig. 3 are more frequent in MNNG survivors.

Whatever the explanation, forcing recombination through the action of MNNG seemed helpful in selecting positively for various mutations including some affecting DNA metabolism. But MNNG essentially allowed the cell to overcome a barrier of heterology insurmountable by homologous recombination pathways under normal conditions. This observation can be extended to other systems taking advantage of the present development of heterospecific gene transfer through episomes – F-primes (Mergeay & Gerits, 1978) or R-primes (Hedges *et al.*, 1977; Van Gijsegem & Toussaint, 1982). Indeed, it is conceivable that genes belonging to unrelated bacteria but controlling the same function would share some homology, antigenic characteristics or evolutionary relationship, but not enough to recombine if put together in the same cross. In bacteria bearing R-prime episomes from various origins, MNNG (or other mutagens) might be helpful in increasing heterospecific recombination between such genes and in producing new hybrid genes.

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