Structure of Recombinants From Conjugational Crosses Between *Escherichia coli* Donor and Mismatch-Repair Deficient *Salmonella typhimurium* Recipients

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ABSTRACT

To get more insight into the control of homologous recombination between diverged DNA by the Mut proteins of the long-patch mismatch repair system, we have studied interspecies *Escherichia coli*/*Salmonella typhimurium* recombination. Knowing that the same recombination pathway (RecABCD) is responsible for intraspecies and interspecies recombination, we have now studied the structure (replacement *vs.* addition-type or other rearrangement-type recombinants) of 81 interspecies recombinants obtained in conjugational crosses between *E. coli* donor and *mutL*, *mutS*, *mutH*, *mutU* or *mut*$^+$ *S. typhimurium* recipients. Taking advantage of high interspecies sequence divergence, a physical analysis was performed on one third of the *E. coli* Hfr genome, which was expected to be transferred to *S. typhimurium* F$^-$ recipients during 40 min before interruption of the mating. Probes specific for each species were hybridized on dot blots of genomic DNA, or on colonies, and the composition of the *rrn* operons was determined from purified genomic DNA. With very few exceptions, the structure of these interspecies recombinants corresponds to replacements of one continuous block of the recipient genome by the corresponding region of the donor genome.

ESCHERICHIA coli and *Salmonella typhimurium* are members of two related bacterial genera which are about 85% homologous at the level of DNA sequence (SHARP 1991). Since they diverged from a common ancestor some 150 million years ago (OCHMAN and WILSON 1987), they have evolved mostly by accumulation of neutral mutations localized predominantly at the third base of codons (CARLOMAGNO *et al.* 1988); thus most, if not all, vital genes functionally complement between these two genera. A comparison of 67 pairs of homologous genes between *E. coli* and *S. typhimurium* indicates that the ratio of the average synonymous substitution rate to the average nonsynonymous substitution rate is approximately 24 (SHARP 1991). Their respective genomes are similar in chromosome size, gene order, and intergenic spacing but differ by 29 "loop" regions, i.e., regions present in only one species (KRAWIEC and RILEY 1990). Yet, in spite of efficient conjugational DNA transfer, there is no significant genetic exchange in crosses between these two bacteria (BARON *et al.* 1968) compared with very frequent exchange observed in intraspecies conjugation. The decrease in exchanges in interspecies crosses was shown to be caused by the DNA sequence divergence which impairs recombination and not due to restriction of incoming DNA (RAYSSIGUIER, THALER and RADMAN 1989; I. MATIC, unpublished observations). Sequence divergence is thought to prevent recombination due to the editing of mismatched recombination intermediates by components of the mismatch repair system (RADMAN 1989; RAYSSIGUIER, THALER and RADMAN 1989; SHEN and HUANG 1989) [for a review on mismatch repair see MODRICH (1991)]. Mismatch repair defective mutants (*mutL*, *mutS*, *mutH*, *mutU*) are partially proficient in interspecies *E. coli*/*S. typhimurium* recombination via the same wild-type recombination pathway (RecABCD) responsible for intraspecies recombination (RAYSSIGUIER, THALER and RADMAN 1989; RAYSSIGUIER, DOHET and RADMAN 1991). SMITH (1991) has reviewed some 45 years of intraspecies conjugation experiments and has concluded that about 80% of the recombinants correspond to replacements of "long chunks" of DNA and 20% to replacements of "short chunks" of DNA, the former recombinant type being dependent on the RecBCD pathway and the latter probably on both the RecBCD and the RecF pathways.

Interspecies *E. coli*/*S. typhimurium* recombination has already been observed in mismatch repair proficient strains, albeit at frequencies from two to six orders of magnitude lower than in comparable intraspecies crosses (BARON *et al.* 1968; JOHNSON *et al.* 1973, 1975; LEHNER and HILL 1985). Most recombinants were true replacements but a significant proportion of them were merodiploids and were shown to correspond to F′, defective F′ and heterospecific duplications (*i.e.*, insertions) within the recipient ge-

nome (BARON *et al.* 1968; JOHNSON *et al.* 1973, 1975; LEHNER and HILL 1985). Many of the non-replacement recombinants had arisen by unequal recombination between *rrn* operons (coding for ribosomal RNA) of one or both bacterial species (LEHNER and HILL 1985). Repetitive sequences such as *rrn* operons have often been implicated in the formation of intraspecies duplications in *S. typhimurium* since analysis of duplications at 38 loci revealed duplications flanked by *rrn* loci (ANDERSON and ROTH 1981). The segment of the genome flanked by *rrnB* and *rrnE* was merodiploid in approximately 3% of rapidly growing bacteria, whereas regions not flanked by the cluster of *rrnD, C, A, B, E, H* operons were duplicated at about hundred-fold lower frequency (ANDERSON and ROTH 1981; SONTI and ROTH 1989). Chromosomal duplications have also been shown to arise by recombination between two *rhs* (*r*earrangement *h*ot *s*pot) sequences (LIN, CAPAGE and HILL 1984) or between REP (*R*epetitive *E*xtragenic *P*alindromic) elements (SHYAMALA, SCHNEIDER and FERRO-LUZZI AMES 1990) of which several hundred copies have been found in the *E. coli* and *S. typhimurium* genomes (DIMRI *et al.* 1992).

Interspecies recombinants between *E. coli* Hfr and *S. typhimurium* F$^-$ described in this report were obtained with different Salmonella recipients (*mutL*, *mutS*, *mutH*, *mutU* or *mut*$^+$) by selecting either for the proximal xylose marker or for distal methionine markers (see Table 2 and RAYSSIGUIER, THALER and RADMAN 1989). This paper presents a physical analysis of some of those interspecies recombinants aimed at answering the following questions: (1) how much *E. coli* donor DNA is acquired by the recombinants, (2) is the *E. coli* donor DNA added to the recipient genome or does it replace the corresponding Salmonella DNA, and (3) is the *E. coli* donor DNA integrated in the recipient Salmonella genome as a single chunk or as dispersed pieces. It appears that the majority of interspecies recombination events are large genomic replacements, as found previously in *mut*$^+$ crosses using genetic methods (BARON *et al.* 1968; JOHNSON *et al.* 1973, 1975; LEHNER and HILL 1985). Thus the inactivation of the mismatch repair system allows for high frequency of interspecies recombination events similar to those obtained in *mut*$^+$ crosses.

MATERIALS AND METHODS

Bacterial strains: Bacterial strains used in this study are *E. coli* Hfr PK3 (*thr-1 lacY1 azi-15 tonA21 supE44*), *S. typhimurium* LT2 F$^-$ SL4213 (*galE496 metA22 metE551 rpsL120 xyl-404* (Fels2) *H1b nml H2-enx ilv-452 hsdSA29 hsdL6*) and interspecies recombinants whose production have already been described (RAYSSIGUIER, THALER and RADMAN 1989).

DNA preparations: Genomic DNA samples were prepared as described (LEHNER, HARVEY and HILL 1984). Plasmid extraction was performed by using the method for isolation of large 60–300 kb plasmids (PUGSLEY and OUDEGA 1987). Preparation of plugs containing genomic DNA for transverse alternating field electrophoresis (TAFE) and electrophoresis was performed as described in RAYSSIGUIER, THALER and RADMAN (1989).

Restriction enzyme digestion: Genomic DNA (*Bam*HI, *Pst*I; New England Biolabs), plasmid DNA (*Bam*HI, *Bgl*II, *Eco*RI, *Hin*dIII, *Pst*I; New England Biolabs) and genomic DNA in plugs for TAFE (*Spe*I; New England Biolabs) were digested as published in HILL and HARNISH (1981), SAMBROOK, FRITSCH and MANIATIS (1989), RAYSSIGUIER, THALER and RADMAN (1989), respectively. Digested samples of genomic and plasmid DNA were separated in 0.6% and 0.5% agarose (SeaKem) gels, respectively.

DNA blotting, probe labeling and hybridizations: Southern blot transfer of DNA was performed as described in SAMBROOK, FRITSCH and MANIATIS (1989). Dot and colony blottings were performed as published previously (ANDERSON and YOUNG 1987; MASON and WILLIAMS 1987, respectively). For all blotting experiments a Hybond-N filter (Amersham) was used. DNA was fixed to the membrane by UV (312 nm) according to the manufacturer's instructions.

All DNA probes used in this work are listed and described in Table 1. DNA probes 0.375–1.2 kb long were labeled with [α-^{32}P]dCTP (3000 Ci/mmol, Amersham) using the Klenow fragment of DNA polymerase I (New England Biolabs) and random sequence oligonucleotide-primer (Promega) as described by FEINBERG and VOLGELSTEIN (1984). Unincorporated [α-^{32}P]dCTP was separated from labeled DNA fragments using a Sephadex G-25 column (medium, DNA grade NAP®-10 Column, Pharmacia), by the method recommended in Pharmacia instructions. Oligonucleotides (27–35 mers, synthesized by the phosphite triester method using a Gene Assembler Plus synthesizer, Pharmacia) used as probes were labeled with [γ-^{32}P]ATP (>5000 Ci/mmol, Amersham; 7000 Ci/mmol, ICN Biomedicals) using the bacteriophage T4 polynucleotide kinase (New England Biolabs) as described in SAMBROOK, FRITSCH and MANIATIS (1989). Unincorporated free [γ-^{32}P]ATP was separated from labeled DNA fragments using spin columns containing Sephadex G-50 fine, DNA grade (NICK Spin Column, Pharmacia), by the method recommended in Pharmacia instructions.

DNA probe 1.2 kb long specific for Escherichia or Salmonella 16S rRNA gene labeled with digoxigenin (DIG) was generated using DIG DNA Labeling Kit (Boehringer), as recommended by Boehringer instruction.

Hybridizations of radiolabeled probes to nucleic acids immobilized on nylon membranes were performed as described in SAMBROOK, FRITSCH, and MANIATIS (1989). Hybridization temperatures (T_{hyb}) were 65° for DNA probes 0.375–1.2 kb long and 60° for oligonucleotides (27–35 mers). The filters with nucleic acids (Southern, dot and colony blots) were prehybridized in 6 × SSC, 5 × Denhardt's solution, 0.5% SDS and 100 μg/ml denatured fragmented salmon sperm DNA, at T_{hyb} for 2 hr. The hybridizations were performed in 6 × SSC, 5 × Denhardt's solution, 0.5% SDS and 100 μg/ml denatured fragmented salmon sperm DNA for 18 hr. For DNA probes 0.375–1.2 kb long filters were washed 2 × 10 min in 2 × SSC, 0.1% SDS at room temperature and 2 × 30 min in 0.1 × SSC, 0.1% SDS at 65°. For oligonucleotide probes (27–35 mers), filters were washed twice for 15 min in 2 × SSC at room temperature and 30 min in 2 × SSC, 0.1% SDS at 60°. Autoradiography was performed by exposure to Fuji RX film with intensifying screens (DuPont), at −80° for one to 5 days.

Hybridization and immunological detection of DIG-labeled probe specific for Escherichia and Salmonella 16S

TABLE 1

DNA probes

Probe specificity	Probe lengths	Probe sequences and localizations	Characteristics	Sources
E. coli rhsB	27 nt*	5′-ATATCGGACACCGACCCCCTGGGTTTA-3′ core extension	*rhsA/B* 33% mismatches *rhsA/D* 41% mismatches *rhsB/D* 33% mismatches	This work[a,b]
E. coli rhsA	27 nt*	5′-GTTACGAATACAGATCCTCTGGGGTTA-3′ core extension	As above	This work[a,b]
E. coli dnaA	27 nt*	5′-TCGCGGCCCCTGCACAGGTGGCGCAAA-3′ about 200 b 3′ from GTG start codon of the *dnaA* gene[c]	*E. coli/S. typhimurium* 48% mismatches	This work[c]
S. typhimurium dnaA	27 nt*	5′-TTGTCGCGCTGCGCAGACAACAACGAC-3′ about 200 b 3′ from GTG start codon of the *dnaA* gene[c]	*S. typhimurium/E. coli* 48% mismatches	This work[c]
E. coli oriC	375 bp**	mini *oriC* 123 b 5′ of DnaA box R1 to 64 b 3′ from DnaA box R4 of mini oriC consensus sequence[e]	*E. coli/S. typhimurium* 15% mismatches	S. BACHELLIER[d]
S. typhimurium oriC	27 nt*	5′-GCCAGCCTTTCAGTTCCGCTTCTATTT-3′ 127 b 3′ of DnaA box R4 of mini oriC consensus sequence[e]	*S. typhimurium/E. coli* > 33% mismatches	This work[e]
E. coli metE-ERIC	35 nt*	5′-TTTTTCTTCCTCTAATTATATGTAAATCCTATGGA-3′ 22 b *metE* + 13 b ERIC	*E. coli/S. typhimurium* > 37% mismatches	This work[f]
S. typhimurium metE-ERIC	35 nt*	5′-TTATTTTATTGAATCTGAAAGTAAAACTCTAAATA-3′ 22 b *metE* + 13 b ERIC	*S. typhimurium/E. coli* > 37% mismatches	This work[f]
rrn operon universal probe	1233 bp**	*E. coli* 16S RNA gene 5′: third base of the *E. coli* 16S RNA gene	*E. coli/S. typhimurium* 4% mismatches	R. CHRISTEN[g]

[a] *rhsD* is at 12 min on the *E. coli* map (see *b*).
[b] DNA sequences from FEULNER *et al.* (1990).
[c] OHMORI *et al.* (1984).
[d] Institut Pasteur, Paris, France.
[e] See MCMACKEN, SILVER and GEORGOPOULOS (1987).
[f] DNA sequences from HULTON, HIGGINS and SHARP (1991).
[g] Observatoire océanologique, Villefranche sur mer, France.
* Synthetic oligonucleotides; ** DNA fragments synthesized by PCR. nt, nucleotide; bp, base pair.

rRNA gene was performed using DIG DNA Detection kit (Boehringer) according to the manufacturer's instructions.

RESULTS

Nature of interspecies recombinants and strategy of analysis: All interspecies recombinants analyzed in this study were obtained in our previous work (RAYSSIGUIER, THALER and RADMAN 1989), in which *E. coli* Hfr donor and *S. typhimurium* F⁻ mismatch repair deficient (*mutL, mutS, mutH, mutU*) or proficient (*mut*⁺) recipient strains were used. The conjugations were carried out for 40 min, the origin of transfer being at 76′–77′ on the *E. coli* map (BACHMANN 1990). The genetic markers used to select for interspecies recombinants were xylose as the proximal one (80′, *i.e.*, within 5′ from the origin of transfer of the chromosome) and methionine (*metE* 86.3′ and *metA* 90.5′; the recipient is a double mutant *metE metA*) as the distal ones (see Table 2). The Hfr donor cells were counterselected by adding streptomycine and by witholding threonine and leucine. The averaged coinheritance of the markers, Met⁺ among the selected Xyl⁺ and Xyl⁺ among the selected Met⁺, was, respectively, in the range 2.5–11% and 2–22%, for the *mut*⁻ crosses and 8% and 6% for the *mut*⁺ cross (RAYSSIGUIER, THALER and RADMAN 1989). We studied two categories of recombinants: 31 Xyl⁺Met⁻ (representing the majority of the selected Xyl⁺ recombinants obtained), and 50 Xyl⁺Met⁺ (26 selected for Xyl⁺ and 24 selected for Met⁺, representing the minority for each selection as indicated above). The 81 recombinants studied are described in Table 2. The Met⁺Xyl⁻ recombinants were not analyzed because our initial intention was to search for a correlation between the stability/instability of the Xyl character, described in the preceding paper (RAYSSIGUIER, THALER and RADMAN 1989), and the structure of the recombinants (*e.g.*, replacement *vs.* merodiploidy). Since the instability was identified only for the Xyl⁺ phenotype (after growth in rich medium and streaking on McConkey xylose plates) we have analyzed only Xyl⁺ recombinants.

Because of the small number of genetic markers used in the preceding study, this analysis employs a number of DNA hybridization probes to determine the nature and the extent of parental DNA exchanges

TABLE 2

Structure of the 81 interspecies *E. coli*/*S. typhimurium* recombinants

				E. coli genetic map[a]														
				rhsB 77′	*xyl* 80.1′	*rhsA* 81′	*dnaA* 83.4′	*oriC* 84.3′	*rrnC* 84.6′	*mutU* 85.9′	ERIC-*metE* 86.3′	*rrnA* 87.3′	*rrnB* 89.7′	*rrnE* 90.5′	*metA* 90.7′	*mutL* 95′	*rrnH* 5.1′	*rrnG* 56.2′	*rrnD* 71.9′
				S. typhimurium genetic map[b]														
S. typhimurium recipient *mut* genotype	Selected marker	Unselected marker	No. of recombinants analyzed	absent	*xyl* 78′	absent	*dnaA* 81′	*oriC* 81′	*rrnc* 82′	*mutU* 84′	ERIC-*metE* 84′	*rrna* 86′	*rrnb* 89′	*rrne* 89′	*metA* 89′	*mutL* 96′	*rrnh* 6′	*rrng* 55′	*rrnd* 73′
mut$^+$	Met$^+$	Xyl$^+$	1	absent	E	E	E	E	E	ND	E	E	E	E	E	ND	S	S	S
	Xyl$^+$	Met$^+$	2	absent	E	E	E	E	E	ND	E	E	E	E	E	ND	S	S	S
	Xyl$^+$	Met$^-$	2	absent	E	E	E	E	E	ND	E	E	E	S	S	ND	S	S	S
	Met$^+$	Xyl$^+$	1	absent	E	E	E	E	E	ND	E	X	S	S	E	ND	S	S	S
	Xyl$^+$	Met$^-$	2	absent	E	absent	S	S	S	ND	S	S	S	S	S	ND	S	S	S
mutL	Met$^+$	Xyl$^+$	2	absent	E	E	E	E	E	ND	E	E	E	E	E	E	S	S	S
	Xyl$^+$	Met$^+$	4	absent	E	E	E	E	E	ND	E	E	E	E	E	E	S	S	S
	Met$^+$	Xyl$^+$	2	absent	E	E	E	E	E	ND	E	E	E	E	E	S	S	S	S
	Xyl$^+$	Met$^+$	3	absent	E	E	E	E	E	ND	E	E	E	E	E	S	S	S	S
	Xyl$^+$	Met$^-$	2	absent	E	E	E	E	E	ND	E	E	E	S	S	S	S	S	S
	Xyl$^+$	Met$^-$	1	absent	E	E	E	E	E	ND	E	E	E	S/S	S	S	S	S	S/S
	Xyl$^+$	Met$^-$	1	absent	E	E	E	E	E	ND	E	E	S	S	S	S	S	S	S
	Xyl$^+$	Met$^-$	1	absent	E	E	E	E	E*	ND	E	E	S	S	S	S	S	S	S
	Xyl$^+$	Met$^-$	1	absent	E	E	E	E	E	ND	E	S	S	S	S	S	S	S	S
	Xyl$^+$	Met$^+$	1	absent	E	E	E	S	S	ND	S	S	S	S	E	S	S	S	S
	Xyl$^+$	Met$^-$	2	absent	E	E	E	S	S	ND	S	S	S	S	S	S	S	S	S
mutS	Met$^+$	Xyl$^+$	4	absent	E	E	E	E	E	ND	E	E	E	E	E	ND	S	S	S
	Xyl$^+$	Met$^+$	5	absent	E	E	E	E	E	ND	E	E	E	E	E	ND	S	S	S
	Xyl$^+$	Met$^+$	1	absent	E	E	E	E	E	ND	E	E	E	S	E	ND	S	S	S
	Xyl$^+$	Met$^-$	3	absent	E	E	E	E	E	ND	E	E	S	S	S	ND	S	S	S
	Xyl$^+$	Met$^-$	1	absent	E	E	E	E	E	ND	E	X	S	S	S	ND	S	S	S
	Xyl$^+$	Met$^-$	2	absent	E	E	E	E	E	ND	S	S	S	S	S	ND	S	S	S
	Xyl$^+$	Met$^-$	1	absent	E	E	E	S	S	ND	S	S	S	S	S	ND	S	S	S
	Xyl$^+$	Met$^-$	1	absent	E	E	S	S	S	ND	S	S	S	S	S	ND	S	S	S
mutH	Met$^+$	Xyl$^+$	8	absent	E	E	E	E	E	ND	E	E	E	E	E	ND	S	S	S
	Xyl$^+$	Met$^+$	4	absent	E	E	E	E	E	ND	E	E	E	E	E	ND	S	S	S
	Xyl$^+$	Met$^-$	3	absent	E	E	E	E	E	ND	E	E	E	S	S	ND	S	S	S
	Xyl$^+$	Met$^-$	2	absent	E	E	E	E	E	ND	E	E	S	S	S	ND	S	S	S
*mutU****	Met$^+$	Xyl$^+$	6	absent	E	E	E	E	E	E	E	E	E	E	E	ND	S	S	S
	Xyl$^+$	Met$^+$	6	absent	E	E	E	E	E	E	E	E	E	E	E	ND	S	S	S
	Xyl$^+$	Met$^-$	6	absent	E	E	S	S	S	S	S	S	S	S	S	ND	S	S	S

The nature of the genetic information given in the columns is deduced from hybridization experiments, except for the *xyl* and *metA* columns for which it is deduced from Xyl$^+$ and Met$^+$ (or Met$^-$) phenotype on minimum medium plates (see RAYSSIGUIER, THALER and RADMAN 1989). For the *mutU* and *mutL* columns, by monitoring the presence of the original transposon (Tn) insertions in the Salmonella *mut* genes, we assessed whether the *E. coli mutL*$^+$ or *mutU*$^+$ genes had replaced the *S. typhimurium* (Tn) genes and we checked by the loss of mutator phenotype (mutation to rifampicine resistance), see RAYSSIGUIER, THALER and RADMAN (1989). The transfer origin of *E. coli* Hfr PK$_3$ is between 76′ and 77′ (BACHMANN 1990). The 7 *rrn* operons are oriented in the following order on the *E. coli* map: *C*, *A*, *B*, *E*, *H* clockwise, and *G*, *D* counterclockwise. E, *E. coli* genetic information; S, *S. typhimurium* genetic information; ND, not done; absent, genetic information not present in *S. typhimurium* or in recombinant genomes; X, hybrid *E. coli*/*S. typhimurium A*/*a* operon (the first letter indicating the 5′ and of the operon) and heterospecific *rrnA* operon duplication (*E. coli rrnA* and *S. typhimurium rrna* operons); S/S, *S. typhimurium rrne*/*d* and *rrnd*/*e* hybrid operon.

* Modified *E. coli rrnC* operon (as described in text).

[a] BACHMANN (1990).

[b] SANDERSON and ROTH (1988).

*** The mean length of the replaced fragment in the *mutU* recipients compared to the mean length of the fragment in the *mutH*, *L*, *S* recipients taken together is significantly smaller ($t = 5.61$ for 24 degrees of freedom; $P < 0.001$).

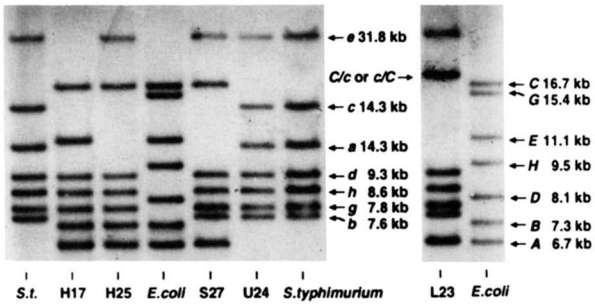

FIGURE 1.—Examples of *rrn* operon analysis. Genomic DNA was digested with *Bam*HI and *Pst*I, blotted and hybridized with an *rrn* operon universal probe, as indicated in the text. *E. coli* and *S. typhimurium* (*S.t.*) are the parental DNAs and the remaining are interspecies recombinant DNAs; the first letter indicates the mutator genotype of the recipient: *mutH*, *mutS*, *mutU* and *mutL*. The size of the different *Bam*HI-*Pst*I fragments coding for rRNA is indicated (as found in LEHNER, HARVEY and HILL 1984). Uppercase letters designate *E. coli rrn* fragments, and lowercase letters designate analogous *S. typhimurium* fragments, *C/c* or *c/C* designates a hybrid fragment (the first letter indicating the 5′ end of the operon). The recombinants correspond to the replacement of respectively four (H17), three (H25), two (S27) and no (U24) *rrn* operons of the recipient by the *E. coli rrn* operons. The L23 recombinant has five *rrn* operons from *S. typhimurium*, one from *E. coli* and one hybrid fragment *C/c* or *c/C* (see E* in Table 2).

in *E. coli* × *S. typhimurium* conjugational crosses. The hybridization probes used are homologous to genes sequenced in both bacteria or repeated sequences present in only one (*E. coli rhsA,B*; *r*earrangement *h*ot *s*pot; FEULNER *et al.* 1990) or in both bacteria (ERIC, Enterobacterial Repetitive Intergenic Consensus; HULTON, HIGGINS and SHARP 1991) (see Table 1). Hybridizations were performed mainly on purified DNA and, for some probes, also on colonies, and the composition of the genomic DNA in *rrn* operons was studied. In order to screen for an extrachromosomal source of recombinant phenotypes, plasmid DNA was analyzed by simple restriction mapping.

Composition of *rrn* operons: The analysis of *rrn* operons allowed us to test for the presence of large duplications (ANDERSON and ROTH 1981) and for putative hybrid *rrn* operons resulting from (unequal) recombination between different *rrn* operons either within or between the two species (LEHNER and HILL 1985). Both bacteria have seven *rrn* operons, five of which are in the region analyzed in our study, located at similar positions, in a clockwise orientation (*C*, *A*, *B*, *E*, and *H*; see Table 2). The more conserved parts of the operons (*i.e.*, the 16S and 23S rRNA genes) show respectively a 5 and 4% divergence between *E. coli* and *S. typhimurium* (R. CHRISTEN, personal communication).

The genomic DNA was extracted, doubly digested with *Bam*HI and *Pst*I (HILL and HARNISH 1981), transferred and hybridized with a universal probe (1.2 kb of the 16S DNA of *E. coli* hybridizing to all of the *E. coli* and *S. typhimurium rrn* operons). The different *rrn* operons were distinguished by the size of the *Bam*HI/*Pst*I fragments they are located on (see Figure 1). We found the replacement of zero, one, two, three or four *rrn* operons of *S. typhimurium* by the corresponding *E. coli rrn* operons, always starting from the same *rrnC* operon (at 84.6′) and progressing clockwise along the genome towards the *rrnE* operon (at 90.5′) (see Table 2 and Figure 1). Only in four out of 81 cases did we find putative hybrid *rrn* operons (LEHNER, HARVEY and HILL 1984).

For the two *mutL* recipient recombinants analyzed, we observed two different situations: in one case, we found two new bands whose sizes (29.5 and 13.7 kb) are compatible with the size of the two hybrid *e/d* and *d/e rrn* fragments (the first letter indicating the 5′ end of the operon); this is consistent with the fact that the parental operons *E* (*E. coli*) or *e* (*S. typhimurium*) and *d* are missing. The origin of the putative hybrid *e/d* fragment is best explained by an intraspecies *S. typhimurium e* × *d* recombination event (as indicated by the *metA* marker) arising independently from, but perhaps coincidently with, interspecies recombination. For the second recombinant (see E* in Table 2 and L23 in Figure 1) obtained with a *mutL* recipient, we did not observe any *C* or *c* operon fragment but instead one new band larger than the *C* operon fragment (see Figure 1). A hybrid *C/c* or *c/C* fragment should be smaller than the *C* operon fragment. One possibility is that a mutation (or a recombination event) eliminated the *Bam*HI site on one side of the *C* operon and that the *Pst*I site on the same side was then used during double *Bam*HI/*Pst*I digestion giving a hybrid *rrn* fragment 2 kb longer than expected (*i.e.*, 18 kb, which is compatible with the observation); other modifications (e.g., insertion of some DNA) are possible. If the apparent loss of the donor *Bam*HI site is due to recombination, it would indicate two crossovers in this region.

For the two other remaining recombinants (one from a *mutS* recipient and the other from a *mut*$^+$ recipient), we have observed a similar complexity at the border between the *E. coli* and *S. typhimurium* DNA. We did not observe an *A* or *a* operon fragment but rather two bands of 8.6 and 16.7 kb (data not shown) whose intensity was greater than the other bands; this result could indicate the presence not only of one *rrnh* and one *rrnC* fragment (see Figure 1) but also of two new fragments of the same size (see *X* in Table 2). The 8.6-kb band could correspond to a hybrid fragment *A/a* and the 16.7-kb band to a fragment containing the two contiguous operons *a* and *A*. One possibility is a recombination between two duplications *a-a* and *A-A* (unequal recombination creating an *A-a* duplication without any *Bam*HI or *Pst*I site between the two, plus one equal recombination creating the hybrid *A/a rrn* fragment). The nature of these putative hybrid *rrn* fragments and of these

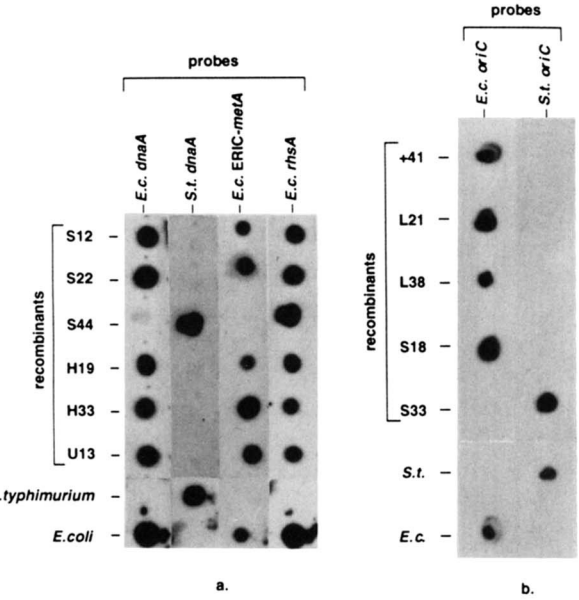

FIGURE 2.—Dot blot (a) or colony (b) hybridizations. Each column represents a DNA probe and each row an interspecies recombinant (except for the two bottom rows which correspond to the parental DNAs); the first letter indicates the mutator genotype of the recipients.

putative local duplications is yet to be determined.

Hybridization on dot blots of genomic DNA: Dot blots of genomic DNA were hybridized with oligonucleotides probing for the presence of *E. coli* or *S. typhimurium* sequences (see Figure 2, panel a, and Table 2). The results of dot blot hybridizations, colony hybridizations (see next paragraph) and of the composition of *rrn* operons were compiled. In conclusion, for most of the recombinants, a region of the Salmonella recipient genome was replaced by the corresponding region of the Escherichia donor genome in one continuous stretch as represented in Table 2. For all the recombinants, the replaced fragment always starts between *rhsB* and *xyl* (*rhsB* was not incorporated in any of the recombinants studied)–*i.e.*, between 77′ and 80.1′ on the map.

For the 31 Xyl^+Met^- recombinants, the replaced fragment ends, for the longest, between *rrnB* and *rrnE*–*i.e.*, between 89.7′ and 90.5′. The extent of the replaced fragment varied according to the recipient mutator gene (see Table 2) as exemplified by the size of the replacements in the *mutU* recipients (the smallest). The mean length of the replaced fragment in the *mutU* recipients is highly significantly smaller than the mean length in the *mutH, L, S* taken together ($t = 5.61$ for 24 d.f.; $P < 0.001$). For the mut^+ recipients, the very small sample precludes any conclusion.

For the Xyl^+Met^+ recombinants, only in the case of the *mutL* recipients (for which we can use the *mutL*::Tn*10* gene of the recipient as a genetic marker) do we have an indication on the limit of the replacement between *metA* and *rrnH* (see Table 2); about one half of the recombinants are $mutL^+$ (donor type). Among the selected Met^+ recombinants only 2 out of 4 had inherited a marker distal to the *metA* gene, *i.e.*, the *mutL* gene of *E. coli*. It may be that the gradient of transfer in interspecies crosses is steeper than in intraspecies cross. In only one case (the second to the last row for *mutL* recipient in Table 2), we found that the replaced fragment did not extend up to the $metA^+$ gene but was shorter, and this recombinant contained an additional local stretch of *E. coli* information at the $metE^+$ and $metA^+$ genes (despite hybridization with the Salmonella ERIC-*metE* probe, the phenotype was Met^+). For the other recipients, one block up to $metA^+$ replaced the Salmonella genome (see Table 2), except for two cases where the replaced fragment stopped at *rrnA* (for mut^+) or *rrnB* (for *mutS*), and there was an additional short stretch of donor information at the *metA* gene ($metA^+$). We found no replacement which extends up to the *rrnH* operon, which again may reflect a steep gradient of transfer.

For 10 recombinants, the recombinant structure was verified by digestion of the genomic DNA with *Spe*I enzyme, pulse field electrophoresis (TAFE) of the fragments (RAYSSIGUIER, THALER and RADMAN 1989) and hybridizations with probes specific for *E. coli* or *S. typhimurium* (including probes specific for the different *rrn* operons of *E. coli* provided by R. CHRISTEN). The results (data not shown) confirmed in all instances the information obtained by other methods.

Hybridization on colonies: To test for the presence of replicons which could allow the extrachromosomal maintenance of the donor information in the recipient, the recombinant colonies were hybridized with an *E. coli* or *S. typhimurium oriC* replication origin sequence. Hybridizations of recombinant colonies with *oriC* probes clearly show the presence of either an *E. coli* or a *S. typhimurium oriC* sequence but not both (see Figure 2, panel b), which is consistent with the lack of any plasmid DNA of the Hfr origin (see next paragraph), as well as with the composition of *rrn* operons and the hybridizations on the genomic DNA which revealed no heterospecific duplications (*i.e.*, no recombinants harbored, for the same locus, extensive sequences from both *E. coli* and *S. typhimurium* parents).

Analysis of plasmids: To test for the possible plasmidic origin of some recombinant phenotypes, extrachromosomal DNA was isolated from diverse recombinant types (3 from *mutL*, 3 from *mutS*, 2 from *mutH*, 1 from *mutU*, 1 from mut^+ recipients) as described by PUGSLEY and OUDEGA (1987) and analyzed by comparing the *Hin*dIII restriction map with that of the natural plasmid of the Salmonella recipient. This natural plasmid has been shown by restriction mapping with enzymes *Hin*dIII, *Bgl*II and *Bam*HI to be identical to the 91-kb pSLT plasmid (TINGE and CURTIS

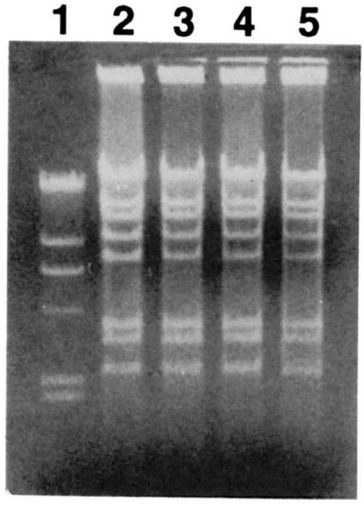

FIGURE 3.—Plasmid *Hin*dIII digestions. Plasmids from *S. typhimurium mut*$^+$ parental strain (column 2) and from interspecies *E. coli/S. typhimurium* recombinants (columns 3 to 5) were isolated, digested with *Hin*dIII restriction enzyme and separated by electrophoresis as described in Materials and methods. Recombinants of columns 3–5, respectively, correspond in Table 2 to the second to last row of *mutL* recipient, the last row of *mutH* recipient (one of the two), and to the first row of *mutU* recipient (one of the six). Column 1 is a size marker (*Hin*dIII digested λ DNA).

1990) which is present in practically all LT2 strains (SANDERSON and STOCKER 1987). Five recombinants analyzed represented the most common type (*e.g.*, only Xyl$^+$ from Escherichia or Xyl$^+$ and *rhsA* from Escherichia or replacement of two, three or four *rrn* operons of Salmonella by the Escherichia *rrn* operons). The other five recombinants included those of specific interest: three with the *dnaA* gene of Escherichia and the *oriC* sequence of Salmonella (one recombinant having an additional local stretch of *E. coli* information at the *metA*$^+$ gene) and two with a putative hybrid *rrn* fragment (*C/c* or *c/C*, *A*, *b*, *e*, *h*, *g*, *d*, and *C*, *X*, *b*, *e*, *h*, *g*, *d*; see Table 2).

For all recombinants, we found only one plasmid whose *Hin*dIII restriction map was identical to the pSLT map (see Figure 3). Thus, it appears that no extrachromosomal replicon bearing donor DNA sequences was present in the recombinants tested. Pulse field electrophoresis analysis of the pSLT plasmid from a number of other recombinants revealed no detectable integration of the donor DNA into the plasmid (results not shown).

DISCUSSION

This physical study of interspecies *E. coli/S. typhimurium* recombinants demonstrates the replacement of one block of the recipient genome by the corresponding donor DNA. Multiple exchanges were observed in only three out of 81 recombinants: in two cases around the *metA*$^+$ marker, and in another case two around *metE*$^+$ and *metA*$^+$ gene, see second to last row for *mutL* recipient in Table 2 where the unselected marker was Met$^+$. The size of the replaced region of the genome varies from short (*xyl* replaced but not *rhsB* and *rhsA*) to a maximum of about 1000 kb (from *xyl* up to, but not including *rrnH*). No recombinants were found to contain the *E. coli rrnH* operon. Our results are compatible with "long chunk" integrations representing the predominant recombination event, as observed in intraspecies crosses (SMITH 1991) and "short chunk" integrations representing a minority. The length of the integrated fragment, however, appears smaller than in intraspecies crosses as concluded from five to six times lower coinheritance of *xyl*$^+$ and *met*$^+$ markers (respectively about ten times lower after selection for the proximal marker and two times lower after selection for the distal marker; RAYSSIGUIER, THALER and RADMAN 1989). We have no compelling interpretation as to why the *mutU* recipients yield smaller replacements than other *mut*$^-$ recipients. The asymmetry observed for the coinheritance of markers (2.5% Met$^+$ among the selected Xyl$^+$ and 22% Xyl$^+$ among the selected Met$^+$; RAYSSIGUIER, THALER and RADMAN 1989) may indicate some regional or marker effect, or reflect the gradient of transfer although the helicase implicated in the transfer is helicase I (MATSON, NELSON and MORTON 1993). For the *mutL* and *mutU* crosses, there could be a potential partial expression of the donor *mut*$^+$ genes. Although we have checked that such partial expression does not affect the frequency of the integration of the proximal marker (xylose), it can not be excluded that the replacement size is affected (RAYSSIGUIER, THALER and RADMAN 1989; our unpublished observations).

The principal conclusion from these results is that the donor *E. coli* DNA was always acquired by the recombinants via a homologous replacement of the Salmonella recipient DNA in the form of a single chunk (with 3 exceptions among 81 recombinants). Merodiploidy of the recombinants was an obvious hypothesis for the instability of Xyl character reported in RAYSSIGUIER, THALER and RADMAN 1989, but our results show that the instability is not related either to the structure of the recombinants (this work) nor to genetic recombination (our unpublished results).

Comparisons with previous studies: Among the 81 recombinants studied we found only two putative heterospecific duplications of the *rrnA* operon (one for a *mut*$^+$ and one for a *mutS* recipient, see *X* in Table 2) and one putative *C/c* or *c/C* hybrid *rrn* fragment. This differs from the finding of 20% merodiploids obtained by selecting for two markers located between *rrnA* and *rrnB* operons in crosses between different *S. typhimurium* donor strains and one *E. coli mut*$^+$ recipient (LEHNER and HILL 1985). Two Hfrs used in that study had an origin of transfer in the same region as the PK_3 Hfr used in our study and had the same

direction of transfer. Of 101 merodiploids characterized, 63 exhibited a novel (*i.e.*, nonparental) *rrn* fragment and, for 51 of them, this hybrid *rrn* operon could explain the establishment of the merodiploidy. However, in the LEHNER and HILL (1985) study, the two selected markers were localized between the 4 *rrn* operons (*C*, *A*, *B* and *E*) which is probably favorable for the formation of heterospecific duplications, whereas in our study the *xyl*$^+$ marker is outside of those 4 *rrn* operons. Such interspecies duplications could occur by circularization of the incoming DNA by recombination between *rrn* genes flanking the marker and perpetuation of the circle as an autonomous plasmid (because of the presence of the *E. coli oriC* origin of replication) or by unequal recombination between non-identical *rrn* genes of both species leading to a duplication by integration into the recipient chromosome. Because of sequence conservation, one can expect a higher fraction of *rrn*-mediated duplications in the *mut*$^+$ than in the *mut*$^-$ background (*i.e.*, there is 5% or 4% divergence for the more conserved 16S and 23S RNA genes as compared to roughly 15% divergence for the rest of the genome). The sample of recombinants originating in the *mut*$^+$ recipients was very small in this study and thus did not allow the detection of merodiploids (we would expect at most one). Since the *metA* gene is flanked by the *rrnH* operon, which was absent from all recombinants, we could not detect any Met$^+$ duplications mediated by recombination between *rrn* operons. Clearly, we can rule out the presence of heterospecific duplications only at the locations where we had probes for both bacteria (see Table 2) or a marker in the recipients with a transposon insert (*mutU* and *mutL*).

Control of horizontal gene transfer: Interspecies recombination has been postulated to occur in the natural history of bacterial genomes (MAYNARD SMITH, DAWSON and SPRATT 1991). Horizontal transfer of genes or parts of genes and their insertion in the recipient chromosome by homologous recombination (replacement) have been described for gram-negative and gram-positive bacterial genera *Neisseria*, *Streptococcus* and *Haemophilus* which have the capacity for uptake and incorporation of extracellular DNA (MAYNARD SMITH, DAWSON and SPRATT 1991; MAZODIER and DAVIES 1991). Even for gram-negative bacteria, such as *E. coli* or *S. typhimurium*, a horizontal transfer of genes has also been invoked for the *pap* and *prs* pili operons (MARKLUND *et al.* 1992) and substantiated for the *phoN* gene (GROISMAN, SAIER and OCHMAN 1992) and for another Salmonella-specific sequence (GROISMAN *et al.* 1993). For the two latter cases, the most likely mechanism of genetic acquisition seems to be conjugation. For SHARP (1991), who studied the *E. coli/S. typhimurium* divergence at the DNA sequence level on 67 homologous protein-coding loci, the lower divergence close to the origin of replication (in the 82′–89′ region of the chromosome) could have resulted from interspecies recombination if recombination between *E. coli* and *S. typhimurium* had occurred subsequent to their divergence from a common ancestor. MEDIGUE *et al.* (1991) in investigating the codon usage of 780 *E. coli* genes suggested that as many as 10% of *E. coli*'s genes might have been inherited by horizontal transmission. They pointed out, moreover, that some *mut* loci themselves (*mutD*, *mutT*, *mutH* and *mutL* (personal communication of A. HENAUT)) seem to have been inherited by horizontal transfer and suggested that the acquisition of "anti-mutator" genes horizontally might actually fix species, raising the possibility that horizontal gene transfer could be involved in *E. coli* speciation. Acquisition of *mutL* and *mutS* genes by a highly polymorphic mutator population could instantly lead to sympatric speciation via the raising of the genetic (reproductive) barriers (RAYSSIGUIER, THALER and RADMAN 1989; RADMAN 1991). In fact, the very identity and the maintenance of species requires that heterospecific gene exchanges be kept to a minimum. ROBERTS and COHAN (1993) have studied sexual isolation in Bacillus using various subgroups of *Bacillus subtilis* and other species (*B. atrophaeus*, *B. amyloliquefaciens* and *B. licheniformis*) and have shown that there is a log-linear relationship between sequence divergence of the *rpoB* gene (estimated by a restriction digest analysis) and sexual isolation. Interspecies transformation frequencies were respectively reduced by factors in the range of 7–400 for a divergence of 0.07–0.14 compared to the transformation frequency of the recipient strain by its own DNA. These large differences in transformation frequencies cannot be explained by the restriction of the incoming DNA or by differential uptake (ROBERTS and COHAN 1993). The structure of the interspecies *E. coli/S. typhimurium* recombinants shows that an efficient horizontal transfer leading to massive chromosomal modifications by homologous recombination can occur under mismatch repair-deficient conditions in a genomic region where the DNA divergence estimated on 16 genes by SHARP (1991) varies from 4.8% to 18.4% (average 12.3%). Therefore, the long-patch mismatch repair system, in particular its mismatch-binding MutS and MutL components which exert the strongest effects on recombination between diverged DNA (RAYSSIGUIER, THALER and RADMAN 1989), probably plays an important role in the maintenance of species, perhaps also in the early stages of the sympatric speciation process.

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