Exchange of Spacer Regions Between rRNA Operons in *Escherichia coli*

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ABSTRACT

The *Escherichia coli* rRNA operons each have one of two types of spacer separating the 16S and 23S coding regions. The spacers of four operons encode $tRNA^{Glu2}$ and the other three encode both $tRNA^{Ile}$ and $tRNA^{Ala1B}$. We have prepared a series of mutants in which the spacer region of a particular *rrn* operon has been replaced by the opposite type. Included among these were a mutant retaining only a single copy of the $tRNA^{Glu2}$ spacer (at *rrnG*) and another retaining only a single copy of the $tRNA^{Ile}$-$tRNA^{Ala1B}$ spacer (at *rrnA*). While both mutants grew more slowly than controls, the mutant deficient in $tRNA^{Glu2}$ spacers was more severely affected. At a frequency of 6×10^{-5}, these mutants phenotypically reverted to faster growing types by increasing the copy number of the deficient spacer. In most of these phenotypic revertants, the deficient spacer type appeared in a *rrn* operon which previously contained the surplus type, bringing the ratio of spacer types closer to normal. In a few cases, these spacer changes were accompanied by an inversion of the chromosomal material between the donor and recipient *rrn* operons. Two examples of inversion of one-half of the *E. coli* chromosome between *rrnG* and *rrnH* were observed. The correlation of spacer change with inversion indicated that, in these particular cases, the change was due to an intrachromatid gene conversion event accompanied by a reciprocal crossover rather than reciprocal exchange between sister chromatids.

THE rRNA operons of *Escherichia coli* comprise a multigene family of similar, but not identical, sequences. The most significant nonhomology is the spacer region separating the 16S and 23S coding regions. Two major types of spacer, one encoding $tRNA^{Glu2}$ and the other encoding $tRNA^{Ile}$ and $tRNA^{Ala1B}$, are found (MORGAN *et al.* 1980). In addition there are two subtypes of $tRNA^{Glu2}$ spacers: depending on the strain, one or two of the $tRNA^{Glu2}$ spacers contain an additional sequence, the *rsl* sequence of unknown function (BROSIUS *et al.* 1981; HARVEY *et al.* 1988). The nonidentity of the various *rrn* operons is not restricted to the spacers, as a degree of divergence is seen in the 16S and 23S coding regions as well (FELLNER, EHRESMANN and EBEL 1970; BOROS, KISS and VENETIANER 1979; CARBON *et al.* 1979; SHEN, SQUIRES and SQUIRES 1982).

The existence of such large but nonidentical homologous genes in a chromosome creates several problems with regard to their interaction and to the maintenance of their diversity. If not otherwise restricted, RecA-dependent recombination between copies could lead to both chromosomal rearrangement and to either the exchange of nonhomologies or to the elimination of diversity altogether. In fact, in both *E. coli* and *Salmonella typhiumurium*, the *rrn* operons are known to interact in ways that lead to a variety of chromosomal rearrangements (reviewed by PETES and HILL 1988). However, in evolutionary terms, both the map positions of the *rrn* operons as well as the identities of the spacers have been remarkably conserved (RILEY and ANILIONIS 1978; LEHNER, HARVEY and HILL 1984). Both *E. coli* and *S. typhimurium* have four $tRNA^{Glu2}$ spacers and three $tRNA^{Ile}$-$tRNA^{Ala1B}$ spacers; furthermore, five of the *rrn* operons have the same spacer association in both species. The significant difference in the respective arrangements is that a reciprocal exchange of spacer types between *rrnB* and *rrnD* has occurred since divergence of the species. In this paper, we examine the frequency and consequences of spacer exchange between *rrn* operons.

MATERIALS AND METHODS

Bacterial mutants and microbial genetic procedures: All bacteria are derivatives of *E. coli* K-12, and their genotypes are given in Tables 1 and 2. The positions and orientations of the *rrn* operons and the positions of other loci used in strain construction are shown in Figure 1. To isolate the *rrnH*-linked *zag-2791*::Tn*10* mutant CH2791, λ1098 infection was first used to produce a number of independent Tn*10* insertion mutants. [The Tn*10* of λ1098 is defective and cannot transpose in the absence of *trans*-complementing functions carried by the vector (MORISATO *et al.* 1983). The Tn*10* mutants were then pooled and used to prepare a phage P1 lysate. This lysate was in turn used to infect a

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TABLE 1

Bacterial strains

Strain No.	Genotype	Source
CH439	F⁻ *trpA36 argH glyS*(low)	HILL, SQUIRES and CARBON (1970)
CH734	F⁻ *trpA36 lysA ilvD130 xyl argH tsx*	COLEMAN, DUNST and HILL (1980)
CH1002	PB153 *rrnD*::[*rrnB-glyT*(Sup)-*rrnE*]	HILL and HARNISH (1982)
CH1045	F⁻ *trpA36 argH glyT*(Sup) *thi purD13 metA zja-1024*::Tn*10 glyV*(ins)	HILL and HARNISH (1981)
CH1070	CH734 *thi purD13 metA zja-1024*::Tn*10*	CH734 × CH1045[a]
CH1097	PB153 *rrnH*::[*rrnB-glyT*(Sup)-*rrnE*]	HILL and HARNISH (1982)
CH2703	F⁻ *trpA36 lysA ilvD130 xyl argH thi purD13 metA tsx*	CH1070 Tet[s]
CH2716	CH2707 In(*rrnG-rrnH*)	This work
CH2780	CH2716 *pheA*::Tn*5* In(*rrnG-rrnH*) *zag-2791*::Tn*10*	This work
CH2791	CD4 *zag-2791*::Tn*10* MetD⁺	This work
CBK001	F⁻ *thy pheA*::Tn*5*	C. BERG
CD4	Hfr PO2A *metB1 proA3 relA1 metD88 lac3 malA36 tsx-76*	W. EPSTEIN[b]
PB153	Cavalli Hfr *trpA36 metB* (λ)	HILL and HARNISH (1982)

[a] Cross conducted by P1 cotransduction with CH1045 as donor.
[b] By way of the *E. coli* Genetic Stock Center.

TABLE 2

***rrn* spacer types**

Strain No.	Spacer types of *rrn* loci[a]							Source or relevant markers
	rrnG	*rrnD*	*rrnC*	*rrnA*	*rrnB*	*rrnE*	*rrnH*	
CH439	g	i-a	g	i-a	g	g	i-a	Table 1
CH1002	g	Both	g	i-a	g	g	i-a	Table 1
CH1097	g	i-a	g	i-a	g	g	Both	Table 1
CH1300	g	Both	g	i-a	g	g	i-a	CH439 *rrnD*::(*rrnB-rrnE*)[b]
CH1300b	g	g	g	i-a	g	g	i-a	CH1300 Sup⁻
CH1472	g	g	g	i-a	g	g	i-a	CH1300b *thr*-101::Tn*10*
CH1473	g	i-a	g	i-a	g	g	g	CH1097 Sup⁻
CH1474	g	g	g	i-a	g	g	g	CH1472 × CH1473 Thr⁺ Met⁺[c]
CH2700	g	g	i-a	i-a	g	g	g	CH1474 revertant
CH2701	g	g	g	i-a	i-a	g	g	CH1474 revertant
CH2702	g	g	g	i-a	g	i-a	g	CH1474 revertant
CH2703	g	i-a	g	i-a	g	g	i-a	Table 1
CH2704	g	i-a	g	i-a	g	i-a	i-a	CH2703 *purD*⁺ *metA*⁺[d]
CH2705	g	i-a	g	i-a	g	i-a	i-a	CH2704 *metB argH*⁺
CH2706	g	i-a	i-a	i-a	g	i-a	i-a	CH2705 *ilvD*⁺[e]
CH2707	g	i-a	i-a	i-a	i-a	i-a	i-a	CH2706 *metB*⁺ *argH*[f]
CH2714	g	i-a	i-a	g	i-a	i-a	i-a	CH2707 revertant

[a] The type of spacer is shown for each *rrn* locus: g, $tRNA^{Glu2}$; i-a, $tRNA^{Ile}$-$tRNA^{Ala1B}$. Transposed duplication mutations are indicated as having both spacers at the insertion site.
[b] Cross conducted by P1 cotransduction with CH1002 as donor.
[c] Cross conducted by conjugation between CH1472 (F⁻) and CH1473 (Hfr).
[d] Cross conducted by P1 cotransduction with CH2702 as donor.
[e] Cross conducted by P1 cotransduction with CH2700 as donor.
[f] Cross conducted by P1 cotransduction with CH2701 as donor.

metD88 mutant, CD4, selecting MetD⁺ (COOPER 1966) and screening for MetD⁺ Tet[r]. Media for bacterial culture, procedures for bacterial crosses and selection for Sup⁺ and Sup⁻ derivatives of *trpA36* mutants were as described (HILL and HARNISH 1982). The *glyT*(Sup) missense suppressor is essentially lethal unless the cell retains a second wild-type copy of *glyT* (HILL, SQUIRES and CARBON 1970). In order to increase the Sup⁻ segregation frequency of transposed duplication mutants, cultures were subjected to a short exposure (10 sec) of UV irradiation from a germicidal lamp under conditions specified previously (HILL and COMBRIATO 1973). Hybrid *rrn* operons are indicated by symbols such as *rrnH/G* which indicates an operon containing the promoter of *rrnH* and the distal portion of *rrnG*.

Analysis of recombinant *rrn* operons: Procedures for DNA extraction, Southern analysis and the labeling of oligonucleotide probes were as specified previously (HARVEY *et al.* 1988). Probes were as follows: specific for *rrn* operons, GCTCCTAGTACGAGAGGA, equivalent to nucleotides 6147–6164 of the *rrnB* sequence (BROSIUS *et al.* 1981); specific for the $tRNA^{Glu2}$ spacer, GTCCTGGGCCTCTAGACGAA, complementary to nucleotides 3237–3256 of the *rrnB* sequence (BROSIUS *et al.* 1981); specific for the $tRNA^{Ile}$-$tRNA^{Ala1B}$ spacer, AGGCTTGTAGCTCAGGTGG, equivalent to the first 19 nucleotides of the $tRNA^{Ile}$ sequence (YARUS and BARRELL 1971). Combined *Bam*HI and *Pst*I restriction digests were used to obtain distinct fragments containing the various *rrn* operons. Wild-type DNA was included on all gels, and the *rrn* operons of the mutants were identified by their alignment with the wild-type bands.

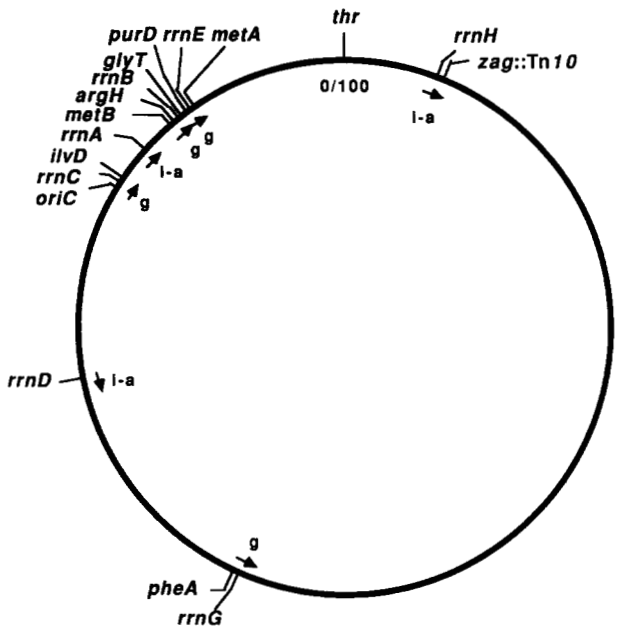

FIGURE 1.—Map of the *E. coli rrn* operons and selected relevant loci (BACHMANN 1987). The 5′ to 3′ polarity of the *rrn* operons is indicated by the arrows. Those operons encoding $tRNA^{Glu2}$ in the 16S-23S spacer are indicated by (g), and those encoding $tRNA^{Ile}$ and $tRNA^{Ala1B}$ are indicated by (i-a).

Hybrid *rrn* operons were identified by a combination of the size of the restriction fragment bearing them and logical deduction from observation of which parental bands were missing (LEHNER, HARVEY and HILL 1984).

Genetic confirmation of the In(*rrnG-rrnH*) mutation: P1 transductions were used to successively introduce *pheA*::Tn*5* (linked to *rrnG*) and *zag-2791*::Tn*10* (linked to *rrnH* into the putative In(*rrnG-rrnH*) mutant, CH2716, to produce CH2780. A P1 lysate of this strain could cotransduce Tet^r and Kan^r back into CH2716 at a frequency of 9.7%, while these markers appeared together only 0.8% of the time when a wild-type strain was used as recipient. This result confirmed that the proximal flank of *rrnG* had become linked to the distal flank of *rrnH* in CH2716. In order to determine the source of the residual Tet^r, Kan^r cotransductants in the control experiment with the wild-type recipient, 41 transductants were further tested by Southern analysis to see if they had acquired the inversion, and all were negative. Presumably the appearance of both Tet^r and Kan^r in these transductants was the result of two independent recombinations at remote positions.

RESULTS

Replacement of the *rrnD* and *rrnH* spacers: An initial objective of this study was to determine if one type of *rrn* spacer could be substituted for another, and, if this could be achieved, to determine the general effect of such substitutions on cell fitness. Mutants with a $tRNA^{Glu2}$ spacer replacing a $tRNA^{Ile}$-$tRNA^{Ala1B}$ spacer were obtained as segregants of mutants isolated in an earlier study (HILL and HARNISH 1982). These starting mutants carried a transposed duplication of the *rrnB-rrnE* chromosomal segment inserted into either *rrnD* (mutant CH1300) or *rrnH* (mutant

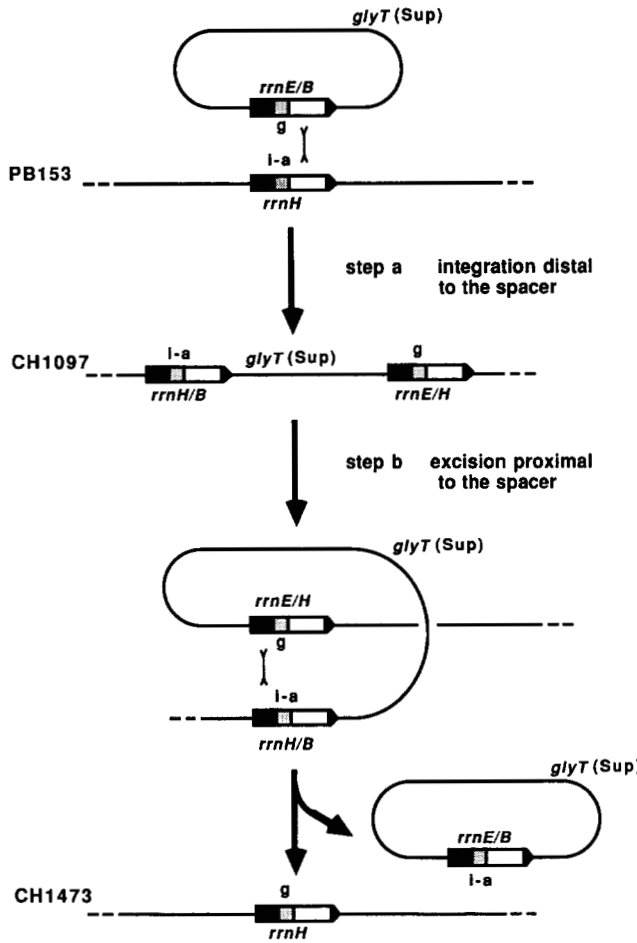

FIGURE 2.—Scheme for replacing the $tRNA^{Ile}$-$tRNA^{Ala1B}$ spacer of *rrnH* with a $tRNA^{Glu2}$ spacer. This construction began with the transposed duplication mutant CH1097 which was derived from PB153 (step a) as described previously (HILL and HARNISH 1982). Reexcision of the transposed segment from the transposed duplication mutant occurs by recombination between the flanking hybrid *rrn* operons (step b). The *glyT*(Sup) missense suppressor mutation that maps to this segment can be used to select for both gain and loss of the duplication. If the integrative crossover occurs in the 23S region distal to the spacer and the excisive crossover occurs in the 16S region proximal to the spacer, the result is the substitution of a $tRNA^{Glu2}$ spacer (g) for a $tRNA^{Ile}$-$tRNA^{Ala1B}$ spacer (i-a) such as was found for mutant CH1473. The 5′ to 3′ polarity of the *rrn* operons is indicated by the arrow; the 16S regions by the filled boxes, the spacer regions by the shaded boxes and the 23S regions by the open boxes. A parallel scheme was used for spacer replacement of *rrnD*.

CH1097) (Table 2). Although these mutants were constructed in several steps, they can be thought of as resulting from the excision of the *rrnB-rrnE* segment, followed by its reintegration into a different *rrn* operon in another chromosome (Figure 2 step a). The unequal crossovers required for this process depend upon the homology of the *rrn* operons. If transposition is into either *rrnH* or *rrnD*, the transposed segment is flanked by hybrid *rrn* operons, one with a $tRNA^{Glu2}$ spacer and one with a $tRNA^{Ile}$-$tRNA^{Ala1B}$ spacer. The relative positions of the spacers depend on whether the integrative crossover occurred in the

16S or in the 23S coding region. Southern analysis of CH1300 [*rrnD::(rrnB-rrnE)*] and CH1097 [*rrnH::(rrnB-rrnE)*] showed that, in both mutants, integration had involved crossover in the 23S region. Consequently, each spacer remained connected to its original promoter. Reexcision of this transposed segment can occur by crossover between the flanking hybrid *rrn* operons. Since the integrative crossover had occurred in the 23S region, excision between the 16S regions would result in the replacement of the original $tRNA^{Ile}$-$tRNA^{Ala1B}$ spacer with a $tRNA^{Glu2}$ spacer (Figure 2 step b). With this rationale in mind, segregants were obtained from each of the two transposed duplication mutants, and the identity of the spacer was ascertained by Southern analysis using spacer-specific probes. One of 12 segregants of CH1300 retained a $tRNA^{Glu2}$ spacer at *rrnD*, and 1 of 9 segregants of CH1097 retained a $tRNA^{Glu2}$ spacer at *rrnH*. The segregants sustaining the spacer substitutions were called CH1300b and CH1473, respectively (Table 2). Each of these segregants had five $tRNA^{Glu2}$ spacers and two $tRNA^{Ile}$-$tRNA^{Ala1B}$ spacers instead of the normal four to three ratio. Simple inspection of colony size gave no indication that this alteration had affected growth.

To test the effect of more extreme alterations of the spacer complement, we introduced the modified *rrnD* and *rrnH* operons into the same strain. To do this, a derivative of the F^- strain, CH1300b, was crossed with the Hfr strain, CH1473, and the recombinants were screened for those that had acquired the *rrnH* allele of the donor, while retaining the *rrnD* allele of the recipient. One of these, CH1474, had a $tRNA^{Glu2}$ spacer at each *rrn* operon except *rrnA* (Table 2). CH1474 did not produce noticeably smaller colonies than control strains such as CH439, but its doubling time in L-broth (supplemented with 0.2% glucose) was significantly longer (22 *vs.* 20 min), presumably because of deficiencies in $tRNA^{Ile}$ and/or $tRNA^{Ala1B}$ levels.

Phenotypic reversion of the single $tRNA^{Ile}$-$tRNA^{Ala1B}$ spacer mutant: If the small growth deficiency observed for CH1474 was related to the low number of $tRNA^{Ile}$-$tRNA^{Ala1B}$ spacer genes, it was reasoned that a mutation increasing the copies of the spacer should lead to faster growth. Accordingly, several independent cultures of CH1474 were subcultured until up to 192 generations had accumulated. The cultures were streaked for single colonies, and the single colonies were screened by Southern analysis. In most cases, the cultures were overgrown with mutants having two *rrn* operons with $tRNA^{Ile}$-$tRNA^{Ala1B}$ spacers. Among 36 independent selections tested, examples were found which had the second $tRNA^{Ile}$-$tRNA^{Ala1B}$ spacer at *rrnB* (9 cases), *rrnC* (6 cases), *rrnD* (7 cases), *rrnE* (8 cases) or *rrnH* (6 cases), but no examples were found where the change involved *rrnG*. Mutants CH2700 ($tRNA^{Ile}$-$tRNA^{Ala1B}$ spacer at *rrnC*), CH2701 ($tRNA^{Ile}$-$tRNA^{Ala1B}$ spacer at *rrnB*) and CH2702 ($tRNA^{Ile}$-$tRNA^{Ala1B}$ spacer at *rrnE* (Table 2) were obtained in this manner.

At least two distinct mechanisms could explain the spacer replacements (Figure 3). One possibility is a reciprocal double crossover between the *rrnA* operon of one chromosome and a different *rrn* operon of a sister chromosome (Figure 3a). This would result in one chromosome with two $tRNA^{Ile}$-$tRNA^{Ala1B}$ spacers, and one with none. These would immediately segregate upon further cell division. The daughter with seven $tRNA^{Glu2}$ spacers and no $tRNA^{Ile}$-$tRNA^{Ala1B}$ spacer would likely not be viable, and in any case, it would not be recovered in this experiment. A second possibility is gene conversion (Figure 3b). In this case, *rrnA* would interact nonreciprocally with one or the other *rrn* operons, causing the replacement of the second spacer without a change in *rrnA*. Gene conversion events could occur by either inter- or intrachromatid interaction. Results to be described below suggest that gene conversion occurs at least occasionally.

Replacement of the *rrnB*, *rrnC* and *rrnE* spacers: The *rrnB*, *rrnC* and *rrnE* operons normally have a $tRNA^{Glu2}$ spacer. However, as described above, reversion of CH1474 provided variants of these three operons with a $tRNA^{Ile}$-$tRNA^{Ala1B}$ spacer. This in turn allowed us to construct mutants deficient in $tRNA^{Glu2}$ spacers. Through a series of P1 cotransductions (Table 2), we constructed a mutant strain CH2707, which had $tRNA^{Ile}$-$tRNA^{Ala1B}$ spacers at all *rrn* operons except *rrnG*.

Phenotypic reversion of a mutant retaining a single $tRNA^{Glu2}$ spacer: The ratio of $tRNA^{Glu2}$ to $tRNA^{Ile}$-$tRNA^{Ala1B}$ spacers in CH2707 was one to six instead of the normal ratio of four to three. It exhibited an easily discernible growth defect, producing noticeably smaller colonies even when compared to cells with only two $tRNA^{Glu2}$ spacers. In addition, its doubling time in supplemented minimal medium was 116 min as compared to 58 min for CH2706, a mutant with two $tRNA^{Glu2}$ spacers. Propagation of CH2707 in L-broth led to rapid overgrowth by large colony-forming variants, and 195 of these were screened by Southern analysis. All were found to have an increased number of $tRNA^{Glu2}$ spacers. The majority of these revertants retained the $tRNA^{Glu2}$ spacer at *rrnG* and acquired a second $tRNA^{Glu2}$ spacer at one of the other six *rrn* operons. The remaining 5% of the large colony formers were tandem duplication mutants. The novel joint of these duplications was a hybrid *rrn* operon with a $tRNA^{Glu2}$ spacer. Consequently, these revertants had a total of two $tRNA^{Glu2}$ and six $tRNA^{Ile}$-$tRNA^{Ala1B}$ spacers.

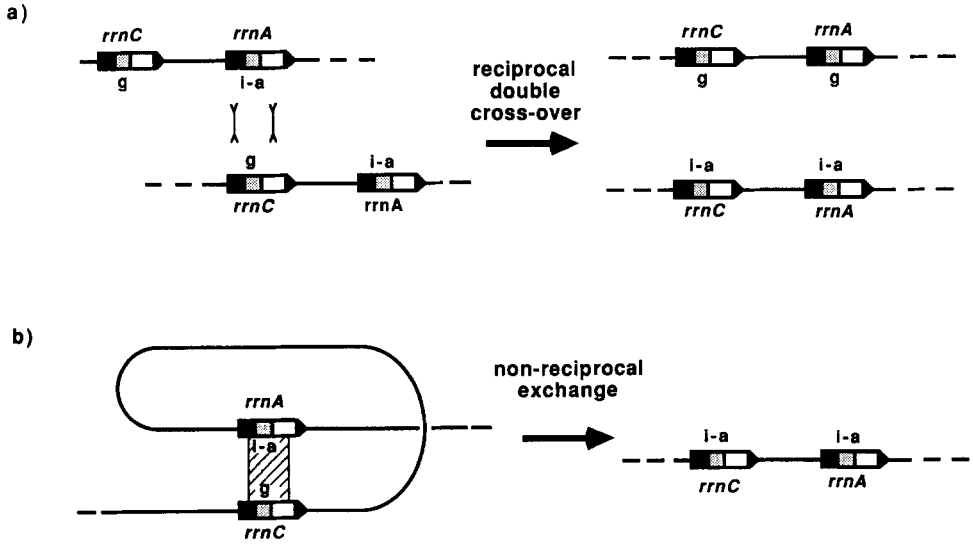

FIGURE 3.—Two possible mechanisms for increasing the number of $tRNA^{Ile}$-$tRNA^{Ala1B}$ spacers in CH1474. a) A reciprocal double crossover between the *rrnA* operon and a different *rrn* operon on a sister chromosome. The consequence of this event would be one chromosome with two $tRNA^{Ile}$-$tRNA^{Ala1B}$ spacers and one with none. b) A nonreciprocal gene conversion between the *rrnA* operon and a second operon on the same chromosome. Crossovers are indicated by X; nonreciprocal exchange is indicated by the hatched box. Other symbols are as in Figure 2.

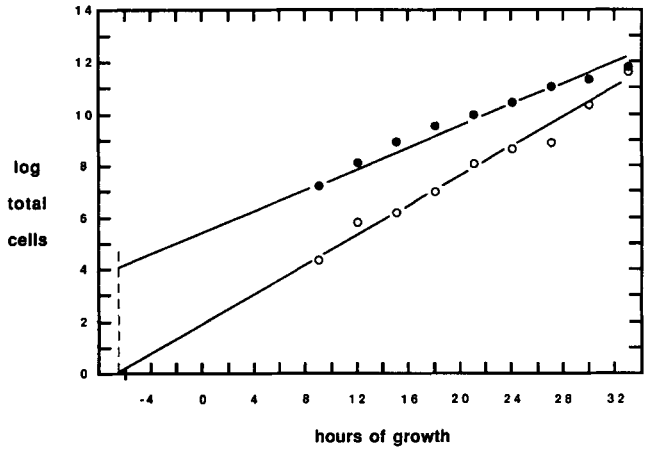

FIGURE 4.—Overgrowth by revertants of a spacer deficient mutant. A colony of CH2707 was suspended in L-broth (supplemented with 0.3% glucose) and maintained in exponential growth by serial dilution. Periodically the culture was sampled, diluted and plated for single colonies. Large (○) and small (●) colony types were counted and their total population was calculated based on the original culture and the accumulated dilutions. The time at which the first large colony-former occurred was estimated by extrapolation and is marked by the broken line. In this experiment, there were 1.1×10^4 small colonly formers in the culture at the time.

Frequency of spacer replacement: The significant difference in size between CH2707 colonies and those of its revertants was used to estimate the frequency of the reversion events. Three colonies of CH2707 were inoculated into separate L-broth cultures and grown continuously in log phase for up to 36 hr. Every 3 hr an appropriate number of cells were plated for counting both small and large colony-forming types. At early times the frequency of large colony formers was too low to be observed. However, at later times large colony formers began to overgrow the culture and their frequency was sufficiently high to be measured. The titers of both types were plotted and the titer of large colony formers at early times was obtained by extrapolation (Figure 4). From the graph, the size of the population at the time the first large colony former appeared could be estimated. The three determinations gave frequency estimates of 9×10^{-5}, 5×10^{-5} and 4×10^{-5}. This relatively high frequency was consistent with a recombinational mechanism of reversion.

Chromosomal inversion occasionally accompanies spacer replacement: As indicated in Figure 3, both reciprocal and nonreciprocal exchange mechanisms could account for the reversion of mutants with altered spacer distributions. The difficulty in determining which mechanism applies stems from the difficulty in detecting both products of an individual crossover (YAMAMOTO *et al.* 1988a). There is one situation, however, in which former partners in a crossover remain associated. That situation occurs when the pairing homologies are inverted repeats within the same chromosome (HILL and HARNISH 1981). A single crossover between them results in an inversion of the intervening material, and the ends of the inversion are the former partners of the crossover. The *rrnG* and *rrnH* operons of *E. coli* are oppositely oriented, so a single crossover between them produces the inversion shown in Figure 5a. We observed examples of CH2707 phenotypic revertants in which the spacer replacement involved *rrnH*. To determine whether inversion between *rrnG* and *rrnH* ever was correlated with this replacement, we isolated a large number of clonally independent revertants of CH2707 and screened them by Southern analysis. From the Southern profiles, we obtained information as to both the numbers and identities of the $tRNA^{Glu2}$ spacers, as well as the identification of hybrid *rrn* operons that are diagnostic of chromosomal rearrangements (see MATERIALS AND METHODS). A total of 21 mutants had experienced events involving *rrnH*; 19 had normal profiles (*i.e.*, no hybrid *rrn* operons), while 2 had profiles that were characteristic of an

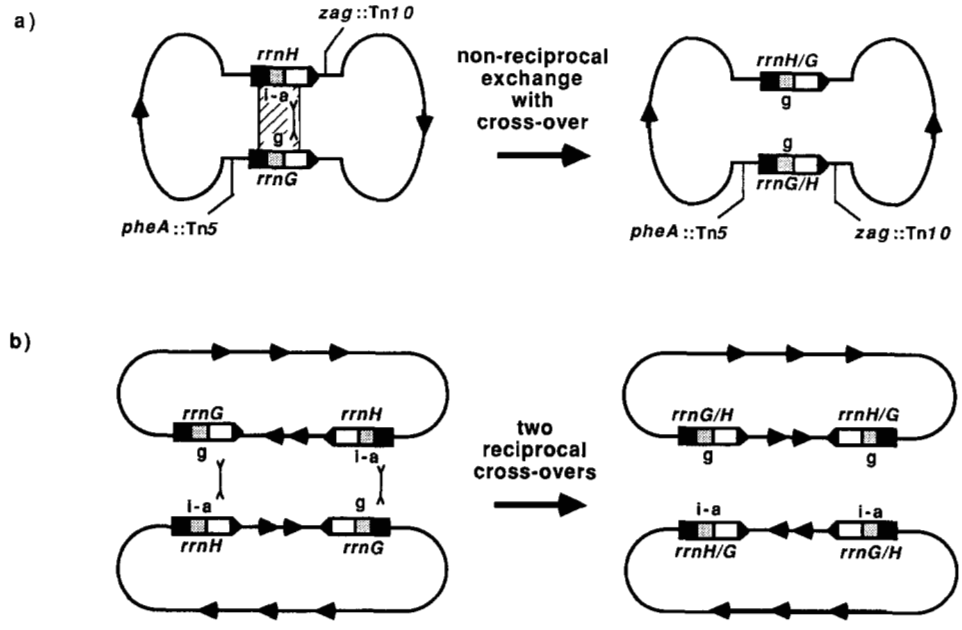

FIGURE 5.—Mechanisms for the replacement of an *rrn* spacer accompanied by chromosomal inversion. a) Gene conversion of the *rrnH* spacer accompanied by reciprocal recombination. In this example, the gene conversion is accompanied by inversion of one-half of the *E. coli* chromosome between *rrnG* and *rrnH*. The ends of the inversion are hybrid *rrn* operons, *rrnG/H* and *rrnH/G*. The positions of *pheA*::Tn*5* and *zag-1291*::Tn*10* are shown. These markers are cotransducted 9.7% when both donor (*e.g.*, CH2780) and recipient (*e.g.*, 2716) are IN(*rrnG-rrnH*). b) A scheme for producing spacer replacement accompanied by inversion through multiple reciprocal crossovers. This is an adaptation of the scheme proposed by YAMAMOTO *et al.* (1988a). Symbols are as indicated in Figures 2 and 3.

inversion between *rrnG* and *rrnH*. Specifically, they lacked bands corresponding to *rrnG* (15.4 kb) and *rrnH* (9.5 kb), but possessed novel bands of the sizes predicted for *rrnG/H* (8.9 kb) and *rrnH/G* (16.0 kb). A $tRNA^{Glu2}$ spacer was present in the hybrid operons at both ends of these inversion mutants. Strain CH2716 was an example of one of these IN(*rrnG-rrnH*) mutants. The presence of the inversion in this mutant was verified genetically (see MATERIALS AND METHODS). As will be discussed below, we believe that this association of spacer replacement with inversion shows that the replacement was through gene conversion.

In this survey of a total of 195 revertants of CH2707, two additional examples of inversion were observed. In both cases the inversion was between *rrnG* and *rrnE*. Significantly, a $tRNA^{Glu2}$ spacer was present in the hybrid *rrn* operons at both ends of the inversion. Thus in the only four inversions encountered, the spacer substitution occurred at the *rrn* operon involved in the inversion, and never in one of the other five uninvolved operons. The appearance of In(*rrnG-rrnE*) mutants among CH2707 revertants was somewhat surprising since this inversion is quite detrimental (HILL and GRAY 1988).

DISCUSSION

In cultures of a mutant retaining its only copy of the $tRNA^{Glu2}$ spacer at *rrnG*, the frequency of spacer conversion of a second operon was estimated to be 6×10^{-5}. This frequency is comparable to other chromosomal rearrangement events involving the *rrn* multigene family in *E. coli*. Deletion between *rrnB* and *rrnE* occurs at a frequency of $1\text{–}2 \times 10^{-4}$ (HILL and HARNISH 1982), while inversion between *rrnD* and *rrnE* occurs at a frequency of about 10^{-5} (HILL and GRAY 1988). Each of these estimates was for an interaction of a particular pair of *rrn* operons with a particular outcome. When all of the possible recombinational interactions between the seven *rrn* operons are considered, the fraction of cells in a population with some change caused by crossovers between *rrn* operons must be substantial. Considering their high frequency, such events must carry a significant selective penalty in nature; otherwise it is difficult to reconcile these results with the observed stability of the enteric bacterial chromosome through evolution (RILEY and ANILIONIS 1978). In the case of mutants in this study which had their complement of one spacer type reduced to a single copy, the mutants were at a rather obvious disadvantage, and overgrowth of cultures by revertants with a more normal ratio of spacers occurred rapidly. Similarly the transposition, duplication and inversion mutations were found to be generally disadvantageous (HILL and HARNISH 1982; HILL and GRAY 1988). However, even the simple exchange of spacers between *rrn* operons not affecting total spacer dosages may be disadvantageous. This is suggested by the similarity of the arrangements in *E. coli* and *S. typhimurium* where five of the operons have the same spacer type in both species, while the other two, *rrnB* and *rrnD*, have the reciprocal arrangement (LEHNER, HARVEY and HILL 1984).

The crossover frequency between *rrn* operons is substantially less than the frequency expected for homologies the size of *rrn* operons (PETES and HILL 1988). At least two factors may contribute to this restriction of crossover. One is the presence of the base substitutions and other non-homologies that distinguish these similar but nonidentical sequences.

Very small degrees of sequence divergence strongly affect RecA-dependent recombination (SHEN and HUANG 1986; WATT *et al.* 1985). The other is the possibility of a physical restriction of access between different homologies.

The excision event described in step b of Figure 2 could occur by crossing over either between the 16S encoding regions proximal to the spacer or between the 23S encoding regions distal to the spacers. Which region was involved could be determined by scoring the spacer type. Of a total of 21 segregants obtained from CH1097 and CH1300, only two occurred by crossover in the proximal regions. In addition, we have determined the crossover position in three of our transposition duplication mutants (HILL and HARNISH 1982) and in the In(*rrnD-rrnE*) mutation present in W3110 (HILL and HARNISH 1981). The crossover in all four of these rearrangements was distal to the spacer. Thus we have observed crossover proximal to the spacer only two times compared to 23 times in the distal portion of the operon. If the crossover frequency depended only on the length of the homology, one-third of these events should have involved the proximal 16S region. Apparently, recombination in the 16S region is more severely restricted than in the 23S region.

With one exception, the selection for spacer replacement produced examples affecting each of the *rrn* operons. The exception was that we never observed a revertant of the single $tRNA^{Ile}$-$tRNA^{Ala1B}$ spacer mutant, CH1474, that had a spacer substitution at *rrnG*. This was out of 36 independent revertants tested. This may have simply been due to chance, but there are other interesting possibilities that should be considered. For example, some structural factor unique to *rrnG* might specifically reduce the frequency of its crossing over with *rrnA*. A second possibility is that less spacer tRNA is produced from *rrnG* with the consequence that mutants with spacer conversion at *rrnG* were less likely to survive the selection. Another possibility is that *rrnG* has an essential function that would be disrupted by spacer substitution. It is tempting to speculate that this might have something to do with the *rsl* sequence. In some strains of *E. coli* K-12, *rrnG* is the only *rrn* operon with the *rsl* sequence in its spacer (HARVEY *et al.* 1988). Perhaps the *rsl* sequence cannot be separated from some other segment of *rrnG* such as the *rrnG* promoter.

Both reciprocal and nonreciprocal recombination can be invoked to explain the substitution of one spacer type for the other (Figure 3). In most cases it is not possible to know from the final result which mechanism applied. However, examples were observed in which the change from one $tRNA^{Glu2}$ spacer copy to two was correlated with an inversion between the apparent donor and recipient *rrn* operons. If it could be established that a single event caused both the spacer replacement and the inversion, it could be concluded that gene conversion of the spacer region occurred. However, there are complications that must be considered before drawing this conclusion. As discussed by YAMAMOTO *et al.* (1988a), this type of product can be produced by two remote, reciprocal crossovers involving two separate chromosomes. One variation of this type of scheme is depicted in Figure 5b. Using a plasmid model system, these workers showed that some apparent gene conversions can be accounted for by multiple rounds of reciprocal crossing over. This kind of scheme is unlikely to account for our observations. As mentioned above, recombination between *rrn* operons occurs at frequencies of 10^{-5} to 10^{-4}. Two independent exchanges would be expected no more frequently than 10^{-8}. However, the mutants combining spacer replacement and inversion occurred much more frequently. Spacer replacements occurred with a combined frequency of about 6×10^{-5}, and about 1% of these were the replacements at *rrnH* with In (*rrnG-rrnH*). We conclude that the hybrid *rrnG/H* and *rrnH/G* were the products of the recombination increasing the $tRNA^{Glu2}$ spacer copy number. More specifically we conclude that gene conversion occurred, in at least these two case. This is of special interest considering the relatively large size, 270 bp, of the nonhomology converted. Gene conversion of a large nonhomology has been observed in yeast (ROEDER and FINK 1982) and in an *E. coli* plasmid (YAMAMOTO *et al.* 1988b).

The relative contributions of reciprocal and nonreciprocal exchange to the observed spacer replacement remains unclear, although two aspects of our observation of CH2707 phenotypic reversion relate to this issue. First, if the assumption were made that 50% of the intrachromosomal gene conversion events were accompanied by reciprocal crossover, the observation of only two inversions among 21 events at *rrnH* would lead us to conclude that only 4 of 21 replacements were due to intrachromatid gene conversion. Consequently, the great majority of replacements would have to be attributed to sister chromatid exchange, either reciprocal or nonreciprocal. However, the results of KLEIN (1984) indicate that, in yeast, intrachromosomal gene conversion between inverted repeats is generally not accompanied by reciprocal crossover. Therefore, this assumption is not warranted, and it is possible that intrachromosomal gene conversion is responsible for much of the spacer replacement. Second, replacement by reciprocal exchange between two *rrn* operons on sister chromatids requires two separate crossovers, one between the 16S and one between the 23S regions (Figure 3a). It was argued in the previous paragraph that two independent ectopic exchanges, involving four *rrn* operons,

would be too infrequent to contribute significantly to the events observed. Extension of this argument would suggest that the two crossovers depicted in Figure 3a, involving two *rrn* operons, is also unlikely, and this would favor a nonreciprocal exchange mechanism (either intra- or interchromatid). However, this extension is not appropriate because we do not know the degree of cooperativity (*i.e.*, negative interference) that might exist between the closely linked exchanges required for replacement by two recirocal exchanges within the same pair of *rrn* operons.

In previous studies of inversion (HILL and HARNISH 1981; HILL and GRAY 1988), it was observed that inversions such as In(*rrnD-rrnE*) and In(*rrnD-rrnB*) which are roughly symmetrical about the origin of replication have only small detrimental effects while the much more asymmetric inversion In(*rrnG-rrnE*) is highly deleterious. Consistent with the idea that the symmetry of the inversion contributes to the degree of the side effects, the large but essentially perfectly symmetrical In(*rrnG-rrnH*) mutation had no obvious effects on cell growth.

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