Spontaneous tandem genetic duplications in *Salmonella typhimurium* arise by unequal recombination between rRNA (*rrn*) cistrons

(gene duplication/chromosomal merodiploidy/transposon)

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Communicated by Sydney Brenner, January 19, 1981

ABSTRACT A method is described to detect and measure the frequency of spontaneous tandem genetic duplications located throughout the *Salmonella* genome. The method is based on the ability of duplication-containing strains to inherit two selectable alleles of a single gene during generalized transductional crosses. One allele of the gene carries an insertion of the translocatable tetracycline-resistance element Tn*10*; the other allele is a wild-type copy of that gene. Using this technique, we have measured the frequency of tandem duplications at 38 chromosomal sites and the amount of material included in 199 independent duplications. These results suggest that, in one region of the chromosome, tandem duplications are particularly frequent events. Such duplications have end points within rRNA (*rrn*) cistrons and probably arise by unequal crossing-over between these dispersed repeated sequences. Spontaneous duplications of this type are harbored by as much as 3% of the bacterial population. Preliminary evidence suggests that such duplications may play a significant regulatory role under conditions of rapid growth. Our analysis has suggested the position on the genome of an additional rRNA cistron.

Recent work has suggested that tandem genetic duplications are frequent mutational events in bacteria and their phages (for review, see ref. 1). Although most estimates of chromosomal duplication frequencies are quite high, considerable variation exists among estimates (ranging from 10^{-5} to 10^{-1} duplications per cell). The diverse techniques used to detect tandem genetic duplications and the peculiarities of individual selections make comparisons among these estimates difficult. To study the duplication process and compare the frequency of duplications at different chromosomal sites, we have developed a method for detecting tandem chromosomal duplications that is applicable to many different loci. This selection is based on the ability of merodiploid strains to inherit two selectable alleles of a single gene. One allele is an insertion mutation of the transposable tetracycline-resistant (Tet^r) determinant Tn*10* (2) into a defined structural gene; the second is a wild-type copy of that gene. Only merodiploid strains can inherit both the insertion (drug resistance) and the wild-type allele (prototrophy). Appropriate transductional crosses detect tandem duplications harbored among the recipient population because of their merodiploid nature. We have used this technique to measure the frequency of spontaneous tandem duplications at 38 sites on the *Salmonella* genome. By a similar technique we have determined the amount of chromosomal material included in 199 independent duplications. The results of these measurements suggest that the frequencies of duplications at different chromosomal loci are indeed highly variable and that the highest frequency of duplications is that for sites located between rRNA (*rrn*) cistrons. Apparently unequal recombination events involving *rrn* se-

quences cause duplications to arise frequently in this region of the chromosome. A preliminary account of this work has appeared elsewhere (3).

MATERIALS AND METHODS

Media and Growth Conditions. The details of media, supplements, and growth conditions have been described (4). Tetracycline and kanamycin were added at 10 μg/ml and 50 μg/ml, respectively.

Bacterial Strains. All strains are derivatives of *Salmonella typhimurium* strain LT2. A nonlysogenizing derivative of the high-transducing phage of Schmieger (5), P22 HT105/1 *int-201*, was used in all transductions. A large number of auxotrophic and fermentation-defective mutants resulting from insertion of the Tn*10* element have been isolated in our laboratory. The sites of insertion have been identified in many of these mutants by up to three independent tests: (*i*) the ability of selected biosynthetic intermediates to fulfill nutritional requirements, (*ii*) a demonstration of transductional linkage of Tn*10* insertions to known genetic markers, and (*iii*) complementation tests between Tn*10* insertions and F′ episomes of known genotype.

Frequency of Tandem Duplications. To measure the frequency of tandem duplications that include particular loci, aliquots of stationary phase cultures of Tn*10*-containing strains were mixed with equal amounts of a P22 lysate of LT2 [2×10^{10} plaque-forming units (pfu) per ml]. Following 30 min of adsorption at 37°C, samples of this mixture were distributed to appropriate selective media. The adsorption mixture was often concentrated by centrifugation or diluted in a minimal-salt solution prior to plating.

Merodiploidy of Tandem Duplications. For the isolation and analysis of independent duplications, single colonies of Tn*5*-containing recipients were inoculated into independent nutrient broth cultures and grown overnight. Wild-type DNA was transduced into these recipients and kanamycin-resistant (Kan^r) prototrophic recombinants were selected. From each culture, a single transductant was picked, purified twice, and verified to be genetically unstable. To determine the amount of material duplicated in these strains, stationary-phase cultures of duplication-containing recipients were spotted onto petri plates that had been seeded with $\approx 2 \times 10^9$ pfu of a selected donor lysate. Donor strains contained Tn*10* insertions into known biosynthetic genes. In parallel experiments, Tet^r, Kan^r recombinants were selected in the presence and absence of the nutritional supplement required by the donor. A comparison of these plates determined unambiguously whether the recipient strain was diploid for the donor Tn*10* insertion site.

Abbreviations: pfu, plaque-forming units; Tet^r and Tet^s, tetracycline resistant and tetracycline sensitive, respectively; Kan^r, kanamycin resistant.
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RESULTS

Isolation of Tandem Duplications. We have developed a technique for detecting tandem genetic duplications that include any of a large number of predetermined sites on the *Salmonella* chromosome. The method involves use of auxotrophic mutations generated by insertion of Tn*10* (2). Such mutants can be used to detect tandem duplications that include the insertion site because of the selectable nature of the insertion element.

Generalized transduction results from the replacement of recipient DNA and genetic markers by those derived from the donor. Thus, when wild-type DNA is transduced into recipients that contain auxotrophic Tn*10* insertion mutations, the majority of prototrophic recombinants simultaneously become tetracycline sensitive (Tet^s) (2). However, among the (predominantly haploid) recipient population are cells containing preexisting

tandem duplications of chromosomal regions that include the Tn*10* insertion site. For this subpopulation, prototrophic transductants remain Tet^r, due to the presence of the second (Tn*10*-containing) copy of the gene involved.

The majority of prototrophic Tet^r recombinants obtained in this manner are concluded to contain tandem genetic duplications by two criteria. (*i*) Such strains are genetically unstable. They segregate auxotrophic Tet^r and prototrophic Tet^s segregant progeny at a high frequency. During nonselective growth of duplication-containing strains, such segregant progeny accumulate in the population. Instability of these transductants is dependent on a functional recombination system. When recA$^-$ mutations are introduced into representative duplication-containing strains, the characteristic instability disappears. (*ii*) Many such strains can be shown to be merodiploid for large regions of their genome (see below). These two criteria (recom-

Table 1. Frequency of tandem duplications in *Salmonella*

Locus	Map position, min	Prototrophic transductants per pfu		Fraction unstable	Duplication frequency
		Tet^s	Tet^r		
thr-557::Tn10	0	2.2×10^{-5}	2.3×10^{-9}	11/12	9.2×10^{-5}
pyrA685::Tn10	1.8	1.3×10^{-5}	7.5×10^{-9}	5/12	2.4×10^{-4}
leu-1151::Tn10	2.8	4.9×10^{-5}	1.6×10^{-8}	9/11	2.9×10^{-4}
pro-662::Tn10	7.0	7.7×10^{-6}	2.7×10^{-9}	ND	3.5×10^{-4}
pyrD2266::Tn10	20.3	6.2×10^{-6}	1.4×10^{-9}	4/12	7.7×10^{-5}
pyrC691::Tn10	22.3	1.1×10^{-5}	1.2×10^{-9}	7/12	6.4×10^{-5}
pyrF696::Tn10	33.4	5.0×10^{-6}	4.0×10^{-10}	ND	8.0×10^{-5}
trp-1013::Tn10	33.9	1.8×10^{-5}	2.5×10^{-9}	5/12	5.8×10^{-5}
aroD553::Tn10	36.0	2.1×10^{-5}	3.8×10^{-9}	9/12	1.4×10^{-4}
hisC8579::Tn10	44.0	2.9×10^{-5}	8.5×10^{-9}	8/12	1.9×10^{-4}
purF1741::Tn10	49.0	3.5×10^{-6}	4.4×10^{-9}	8/12	8.7×10^{-4}
cysA1539::Tn10	52.0	3.6×10^{-6}	1.1×10^{-9}	7/12	1.8×10^{-4}
purC882::Tn10	53.6	3.0×10^{-6}	3.0×10^{-9}	12/12	1.0×10^{-3}
purI1757::Tn10	54.1	3.3×10^{-6}	2.1×10^{-9}	11/12	5.9×10^{-4}
guaB544::Tn10	54.4	3.3×10^{-6}	1.0×10^{-9}	10/12	1.5×10^{-4}
guaA544::Tn10	54.4	8.3×10^{-6}	2.6×10^{-9}	8/12	2.1×10^{-4}
glyA540::Tn10	56.5	2.7×10^{-5}	9.9×10^{-9}	10/12	3.1×10^{-4}
purG1739::Tn10	56.6	3.0×10^{-5}	1.3×10^{-8}	8/12	2.8×10^{-4}
tyrA555::Tn10	58.3	1.5×10^{-5}	2.3×10^{-8}	11/12	1.4×10^{-3}
pheA554::Tn10	59.0	1.2×10^{-5}	2.1×10^{-8}	10/12	1.4×10^{-3}
cysC1511::Tn10	60.0	7.6×10^{-6}	1.3×10^{-8}	10/11	1.6×10^{-3}
argA1832::Tn10	61.3	9.1×10^{-6}	1.1×10^{-8}	6/12	6.0×10^{-4}
lys-565::Tn10	61.9	1.2×10^{-5}	5.1×10^{-8}	12/12	4.2×10^{-3}
serA977::Tn10	62.7	1.3×10^{-5}	3.7×10^{-8}	10/12	0.3×10^{-3}
metC1975::Tn10	66.0	4.8×10^{-6}	2.1×10^{-9}	12/12	4.4×10^{-4}
argG828::Tn10	67.7	6.5×10^{-6}	4.3×10^{-9}	6/12	3.3×10^{-4}
cysG1510::Tn10	72.4	1.6×10^{-5}	4.5×10^{-8}	10/11	2.5×10^{-3}
ilv-2104::Tn10	83.0	1.4×10^{-5}	1.1×10^{-7}	12/12	7.8×10^{-3}
metE862::Tn10	84.0	7.5×10^{-6}	6.2×10^{-8}	11/12	7.5×10^{-3}
metB869::Tn10	88.2	5.8×10^{-6}	1.9×10^{-8}	11/12	3.0×10^{-3}
metF877::Tn10	88.2	7.2×10^{-6}	4.6×10^{-8}	11/11	6.3×10^{-3}
argH1823::Tn10	88.9	6.9×10^{-6}	7.1×10^{-8}	12/12	1.0×10^{-2}
purD1735::Tn10	90.0	4.5×10^{-6}	1.2×10^{-7}	12/12	2.6×10^{-2}
purH887::Tn10	90.0	2.9×10^{-6}	9.6×10^{-8}	12/12	3.2×10^{-2}
met900::Tn10	90.1	5.1×10^{-6}	3.5×10^{-8}	12/12	6.8×10^{-3}
purA874::Tn10	93.0	7.8×10^{-6}	2.2×10^{-9}	9/12	2.1×10^{-4}
pyrB692::Tn10	97.8	6.3×10^{-6}	1.2×10^{-8}	10/11	1.7×10^{-3}
serB965::Tn10	99.6	1.4×10^{-5}	9.3×10^{-9}	11/12	6.1×10^{-4}

Wild-type DNA was transduced into auxotrophic recipient strains containing the indicated Tn*10* insertion mutations. All gene designations and map positions are those of the revised *Salmonella* genetic map (6). An approximate measure of the frequency of duplications of each Tn*10* site is obtained by comparing the efficiency of transduction (transductants per pfu) selecting prototrophy to the efficiency of transduction selecting Tet^r and prototrophy. In each case, a representative number of Tet^r prototrophic recombinants were picked, purified, and tested for stability. Only strains exhibiting such instability were concluded to contain tandem duplications. These results are indicated as "fraction unstable." The frequency of duplication for each locus was calculated by multiplying the fraction of prototrophic recombinants that are Tet^r by the fraction of Tet^r prototrophs that are genetically unstable.

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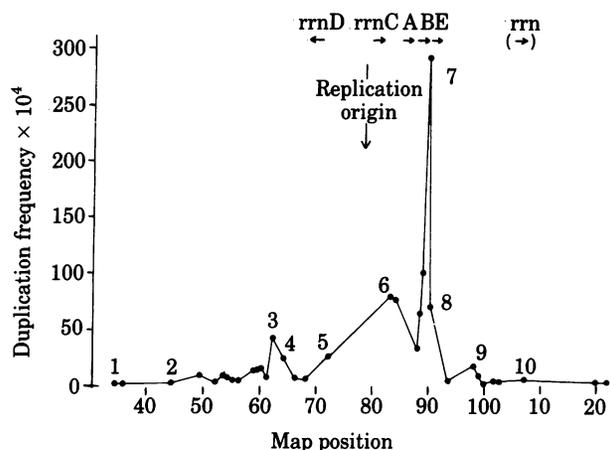
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FIG. 1. Frequency of spontaneous tandem duplications at points on the *Salmonella* chromosome. Each point represents one of the 38 loci whose frequencies of duplication are shown in Table 1. A number of particular loci are indicated, as are the origin of chromosomal replication and the positions and relative orientations of the several rRNA (*rrn*) cistrons. We tentatively assign the locus *rrnH* to the *Leu-Pro* interval. Positions: 1, *trp*; 2, *his*; 3, *lys*; 4, *ser*; 5, *cysG*; 6, *ilv*; 7, *purD*; 8, *metA*; 9, *pyrB*; 10, *pro*.

bination-dependent instability and chromosomal merodiploidy) are characteristic of and diagnostic for strains containing tandem duplications (1).

Frequency of Tandem Duplications. The spontaneous frequency of tandem duplications can be estimated for each Tn*10* insertion site by comparing the frequency of recombinant types involving rare duplication-containing recipients with the frequency of recombinants involving the total recipient population. The difference between these frequencies is a measure of the fraction of the recipient population that harbors a tandem duplication. Transduction events involving the total population are measured as the efficiency of transduction (transductants per pfu) while selecting only prototrophy. Events involving the duplication-containing subpopulation are measured as the frequency of prototrophic recombinants that are also Tet^r. Several tests suggest that the presence of Tn*10* in recipient cells does not alter the normal duplication frequency.

The results of these measurements for 38 chromosomal sites are shown in Table 1 and Fig. 1. Also indicated in Fig. 1 are the positions and relative orientations of the several rRNA (*rrn*) cistrons (7) and the origin of replication (8) for the *Escherichia coli* chromosome. Because the *Salmonella* and *E. coli* genetic maps are virtually identical (6, 9), we assume that these loci are similarly located in *Salmonella*. The frequency of spontaneous duplications is remarkably high throughout the region of the chromosome bounded by the directly repeated *rrn* cistrons. The peak of this curve suggests that spontaneous duplications of *purD* and *purH* are harbored by 3% of the recipient population! Regions of the chromosome that are not bounded by the cluster of *rrn* cistrons have duplication frequencies generally between 10^{-4} and 10^{-3} per cell.

Several tests suggest that the frequency of duplications we observe is not affected by the act of transduction or by the presence of Tn*10* in recipient cells. We are directly measuring the proportion of duplications among the recipient population. (*i*) The observed duplication frequency does not depend on which of several different wild-type donor lysates is used for the transduction. (*ii*) The duplication frequency determined for the histidine operon agrees within a factor of two with an independent estimate obtained by techniques that do not involve use of Tn*10* (4). Furthermore, the frequency of *his* duplications measured

by this technique is unaffected by the presence of Tn*10*. (*iii*) The duplication frequencies of representative loci have been measured using crosses in which Tn*10* was present in the donor, rather than the recipient. In the four cases tested, the measured frequencies were approximately equal to those given in Table 1. (*iv*) The duplication frequencies for several loci have been determined by using a different translocatable drug-resistant element (Tn*5*, Kan^r; ref. 10). Its use yields estimates of duplication frequencies that agree closely with those in Table 1.

Merodiploidy Harbored by Independent Duplications. The surprisingly high frequency of tandem duplications in certain chromosomal regions suggests that *rrn* cistrons are participating in the duplication process. Tandem duplications that include the histidine operon have previously been shown to occur by a recombination-dependent process (4). Thus, an attractive hypothesis is that *rrn* cistrons provide homology at separated sites on the chromosome and serve as points for unequal crossing-over. Such exchanges yield deletions (probably lethal) and duplications of the intervening regions. This hypothesis predicts that (*i*) duplication formation is dependent on recombination and (*ii*) discrete chromosomal regions are included in the duplications. Specifically, such duplications would be expected to have end points at *rrn* loci and duplicate all markers in the intervening region. Unfortunately, the first of these predictions is not easily tested. Recipient cells must be recombination proficient to participate in the transduction events necessary to detect the duplications by the methods described.

The second of these predictions can be tested. We have mapped duplications and determined whether their end points coincide with sites of known *rrn* loci. To do this, a new set of duplications was selected using auxotrophs made by insertion of Tn*5* (Kan^r). The Tn*5* auxotrophs were used as recipients in transduction crosses. Selection was made for prototrophy (*Aux*$^+$) and Kan^r (*aux*::Tn*5*). Only strains carrying a duplication could maintain both alleles. (This procedure is identical to that used with Tn*10* to estimate duplication frequencies.) The resulting duplications (*aux*$^+$; *aux*::Tn*5*) were then mapped by using Tn*10* auxotrophs as donors. Transduction crosses were performed by using the prototrophic duplication strains as recipients and various Tn*10* auxotrophs as donors. Selection was made for prototrophy, Tet^r, and Kan^r. If the recipient duplication included the gene corresponding to the donor Tn*10* insertion, then prototrophic Tet^r transductants arose with high frequency. If the recipient had only one copy of this gene, selection for Tet^r seldom yielded prototrophic recombinants. By performing such crosses with a variety of Tn*10* auxotrophs, the extent of the duplications of the recipients were mapped.

Duplications were selected at 10 sites in the chromosome (sites for which Tn*5* insertion auxotrophs were available). The results of mapping 199 of these duplications are shown in Figs. 2 and 3. All duplications described in these figures are of independent origin.

Fig. 2 shows the results of mapping 115 duplications selected to include the *ilv*, *metF*, or *purD,H* loci. All of these genes are known to be located between *rrn* loci and show a very high frequency of duplication. With only one exception, both end points of all 115 duplications lie in a map interval known to include a *rrn* locus. Also presented in Fig. 2 and 19 deletions obtained by selection for duplication of the *thr* or *leu* loci (see below). Fig. 3 shows the extent of 65 additional duplications selected to include the *pyrD*, *trp*, *his*, *cysA*, and *cysG* loci.

Several aspects of the data in Figs. 2 and 3 merit attention. (*i*) For the loci showing a high duplication frequency, 114 out of 115 duplications have both end points at *rrn* cistrons. This suggests that unequal recombination between these sequences occurs frequently and causes the high duplication frequency

FIG. 2. Merodiploidy harbored by 134 independent duplications. The *Salmonella* genetic map from *cysG* (min 72) to *pro* (min 7) is shown. The region from 80 min to 95 min is expanded. Duplications have been selected to include either *ilv*, *metF*, (*purB,H*), *thr*, or *leu*. These duplications have been tested for inclusion of each of the 17 genetic markers indicated above the bold line. The markers represent known points of insertion of Tn*10*. The amount of material duplicated is depicted by the horizontal bars below the bold line. The loci used for selection are indicated by the dots on the horizontal bars. The number of multiple identical isolates is indicated beside each bar. Also indicated are the positions and relative orientations of the rRNA cistrons *rrnA, B, C, E*, and the tentatively assigned *rrnH*.

seen. (*ii*) Of 19 duplications selected for the *thr* and *leu* loci, 13 have a left end point in an interval containing an *rrn* locus and a left end point between *leu* and *pro*. This suggests that an additional *rrn* locus may be located between *leu* and *pro*. We believe that an additional *rrn* cistron will be found in this region and have tentatively designated this cistron *rrnH*. (*iii*) At many loci, particular duplication types have occurred repeatedly. This is consistent with the existence of homologous sequences at distinct points on the chromosome. These sites may recombine to generate duplications. (*iv*) At each locus, the shorter duplication types arise more frequently; it is possible that homologous sequences are more likely to recombine if placed closer together in the chromosome. Overlapping duplications that together include nearly the entire chromosome have been isolated. A single area of the chromosome has unambiguously *not* yielded duplications (see Fig. 3). This region is located generally between *pyrC* and *his* (excluding *trp*). The termination site for chromosomal replication (11) is known to lie within this region. We suspect that our failure to recover duplications of this region is due to their lethal nature. It is uncertain whether we have isolated strains that duplicate the origin of chromosomal replication (8). Although many of our duplications have end points

within the appropriate chromosomal region (*cysG–ilv*), none of them spans the entire region. Thus, we do not know whether all sequences have been included.

The very high frequency of spontaneous tandem duplications that include rRNA cistrons has prompted us to consider what role (if any) such duplications play in normal cell growth and physiology. Specifically, we have considered the adaptive significance of duplication (and perhaps amplification) of particular chromosomal sites or regions as a function of certain growth parameters. One observation suggests that gene duplication may play a significant regulatory role: the frequency of duplications involving the *rrn* loci is influenced by previous growth conditions. The duplication frequencies given in Table 1 were determined for cells grown in rich medium at a fast doubling time. However, if cells are grown slowly in a minimal-salt medium (for ≈30 generations), the duplication frequencies for those events that involve rRNA cistrons are much less (one-tenth to one-fourth). These data are shown in Table 2. Duplications that do not involve rRNA cistrons are generally unaffected by prior growth conditions. Thus, rapid growth appears to affect specifically the observed frequency of *rrn*-type duplications.

FIG. 3. Merodiploidy harbored by 65 independent duplications. The *Salmonella* genetic map from *thr* (min 0) to *ilv* (min 83) is shown. Duplications have been selected to include either *leu, pyrD, trp, his, cysA*, or *cysG*. These duplications have been tested for inclusion of each of the 29 genetic markers indicated above the bold line. The markers represent known points of insertion of Tn*10*. The amount of material duplicated is depicted by the horizontal bars below the bold line. The loci used for selection are indicated by the dots on the horizontal bars. The number of multiple identical isolates is indicated beside each bar.

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Table 2. Effect of growth conditions on duplication frequency

Locus	Map position, min	Duplication frequency	
		Broth grown	Minimal grown
leu	2.8	2.9×10^{-4}	3.1×10^{-4}
trp	33.9	5.8×10^{-5}	6.2×10^{-5}
his	44.0	1.9×10^{-4}	1.6×10^{-4}
purG	56.6	2.8×10^{-4}	1.5×10^{-4}
lysA	61.9	4.2×10^{-3}	3.8×10^{-4}
ilv	83.0	7.8×10^{-3}	1.9×10^{-3}
metE	84.0	7.5×10^{-3}	1.4×10^{-3}
metB	88.2	3.0×10^{-3}	1.1×10^{-3}
metF	88.2	6.3×10^{-3}	1.1×10^{-3}
argH	88.9	1.0×10^{-2}	2.3×10^{-3}
purD	90.0	2.6×10^{-2}	3.4×10^{-3}
purH	90.0	3.2×10^{-2}	9.5×10^{-3}
metA	90.1	6.8×10^{-3}	5.6×10^{-4}
purA	93.0	2.1×10^{-4}	1.9×10^{-4}
pyrB	97.8	1.7×10^{-3}	1.1×10^{-4}

Observed frequency of duplications depends on prior growth conditions. Transductions were performed by using the same methods and strains as shown in Table 1, and the data shown for nutrient broth grown cultures are taken from Table 1.

DISCUSSION

Spontaneous tandem duplications are frequent mutational events throughout the *Salmonella* chromosome; the frequency of cells carrying a duplication of a particular site ranges from approximately 10^{-4} to 3×10^{-2}. This wide variation in frequencies of duplications measured at different chromosomal sites is due to the very frequent occurrence of specific types of duplications. Duplications are frequent in chromosomal regions that contain rRNA cistrons and probably arise by unequal crossing-over between these dispersed repeated sequences. Our results confirm the observations of Hill and coworkers (12, 13) concerning the involvement of rRNA cistrons in tandem duplication of the *E. coli glyT* locus.

Several lines of evidence suggest that *E. coli* contains seven rRNA operons (for review, see ref. 7). To date, the locations of six of these cistrons have been identified. Our analysis has suggested the position of the seventh (and perhaps final) rRNA cistron on the *Salmonella* and *E. coli* chromosomes. Thirteen independent duplications have been isolated that have one end point at either *rrnA*, *rrnB*, or *rrnE* and their second end point between *leu* and *pro*. We thus suggest that the *leu–pro* interval may contain another as yet unidentified rRNA cistron and tentatively assign the locus *rrnH* to this region.

The mechanism for the variation of duplication frequency with growth rate is unknown, but several possibilities are being entertained. Duplications occur by spontaneous recombination between *rrn* loci. Under rapid growth conditions, cells harboring such duplications achieve a higher doubling time and increase in frequency in the population. The selective advantage of duplication-containing cells could be due to an increase

in gene dosage of rRNA cistrons themselves. It is likely that growth rate in rich medium is limited by the supply of ribosomes [for review, see Nierlich (14)]. Alternatively, amplification of genes found within the duplicated region may be responsible for this selective advantage. It is interesting to note that within the frequently duplicated *rrnB–rrnE* interval are the genes for the B and B' subunits of RNA polymerase (15, 16) and one of the two genes for elongation factor Tu (17). The *in vivo* rate of synthesis of core polymerase is limited slightly by the rate of synthesis of the B and B' subunits (for review, see ref. 18). Elongation factor Tu is one of the most abundant proteins in *E. coli*, accounting for as much as 5% of total protein (19). A gene dosage increase in any of these genes might have significant effects on cell growth. Another explanation for amplification of the *rrn* regions may be that under rapid growth conditions, multiple chromosomal replication forks provide increased dosage of the *rrn* loci near the origin. This provides enhanced opportunity for recombination, causing the duplication frequency to increase. It is also possible that a specific or general recombination mechanism may exist that generates duplications in response to some metabolic signal.

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