HIGH FREQUENCY OF GENETIC DUPLICATIONS IN THE *dnaB* REGION OF THE *ESCHERICHIA COLI* K12 CHROMOSOME

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Manuscript received March 25, 1981
Revised copy received June 22, 1981

ABSTRACT

The region that includes the *dnaB* locus on the *E. coli* K12 chromosome was shown to be duplicated at high frequency in cell populations. The duplications were shown to be arranged in tandem and segregated at various frequencies. Segregation was dependent on the *recA* recombination system, but independent of *recB,C*. Though most of the data was obtained with *dnaB*::Tn*10* insertion mutants, the duplications were shown to occur in the absence of Tn*10*.

CONSIDERABLE evidence has accumulated that organisms like *E. coli*, *S. typhimurium* and *S. coelicolor* often contain duplicated regions within their chromosomes (HOPWOOD 1967; STODOLSKY 1974; HILL *et al.* 1977; ANDERSON and ROTH 1978). These duplications can arise from unequal sister chromosome exchanges *via* legitimate or illegitimate recombination (ANDERSON and ROTH 1977). Certain regions of the chromosome exhibit a higher frequency of duplication than others, possibly because of the location of homologous sequences bracketing these segments (ANDERSON and ROTH 1978).

During a study of *dnaB*::Tn*10* insertion mutations, we observed tandem duplications of the *dnaB* region of the *E. coli* chromosome. The criterion used to established the presence of a duplication was the segregation of duplicated, scorable alleles. Though most of the duplications contained a *dnaB*$^+$ and a *dnaB*::Tn*10* allele, as this combination was easily analyzed, duplications were also obtained using a *dnaB*$^+$ and temperature-sensitive (ts) *dnaB* combination. The duplication-bearing strains and the resultant haploid segregants were distinguished by straight forward growth tests.

In addition to showing that duplications of this region of the chromsome occur often (frequencies of 6–60% depending on the method of measurement), evidence is presented that segregation is dependent on the bacterial *recA* recombination function.

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MATERIALS AND METHODS

Bacterial and bacteriophage strains: All bacteria were *Escherichia coli* K12 (Table 1). Phages P1*Cm-1bac-1* $r^-_{P1}m^-_{P1}$ and P1*Cm-1bac-1ban-1* were obtained from M. YARMOLINSKY and will be referred to as P1*bac* and P1*bac ban* for simplicity. The generalized transducing phages: P1*vir*^{s} was from our collection; T4GT7 was obtained from J. S. Parkinson. λ*c1*60 and λ*vir* were from our collection. λ*c1*60 *dnaB*+ was constructed in this laboratory from a λ*c1*857 *dnaB*+ phage (SCLAFANI, WECHSLER and SCHUSTER 1981).

Media and antibiotics: M9 minimal media, P1 medium, nutrient and solid media were as previously described (WECHSLER and KURTZ 1979). Chloramphenicol and tetracycline were used in solid media at final concentrations of 15 μg/ml each, and methyl methane sulfonate at 0.02%.

Genetic techniques: Conjugations and transductions were conducted as described (WECHSLER and GROSS 1971; WILSON *et al.* 1979). Isolation of P1 lysogens was performed according to D'ARI *et al.* 1975. P1*bac* lysate preparation by induction with mitomycin C has been described (TOUATI-SCHWARTZ 1979; SCLAFANI, WECHSLER and SCHUSTER 1981).

Cryosensitivity (Cry$^-$) and λ growth: Colonies were either streaked or replica plated on nutrient plates at 25° to assay for cryosensitive growth. λ growth was assayed by the agar overlay method and checking for plaque formation *dnaB*::Tn*10* (P1*bac*) strains support growth of λ*c1*60 *dnaB*+, but not of λ*c1*60.

RESULTS

Isolation of dnaB *duplications*

We have isolated several *dnaB*::Tn*10* insertion mutations (SCLAFANI, WECHSLER and SCHUSTER 1981). Since *dnaB* is an essential gene for bacterial DNA replication (WECHSLER 1978), *dnaB*::Tn*10* mutations can exist in a haploid strain only if the strain is lysogenic for P1*bac*, which supplies a *dnaB* analogue protein encoded by the *ban* gene of the prophage. These *dnaB*::Tn*10*, P1*bac* lysogens are cryosensitive (Cry$^-$) for growth and cannot support growth of

TABLE 1

Bacterial strains

Strain	Relevant genotype	Source or reference
594	*sup*+ *rpsL*179	WEIGLE 1966; J. S. PARKINSON
RS116	594 *dnaB518*::Tn*10*(P1*bac*)	SCLAFANI, WECHSLER and SCHUSTER 1981
JW165	*malB45*	*polA*+ derivative of JW164 (LARK and and WECHSLER 1975)
RS121	594 *malB45*	Transduction
RS113	F'118 *ampA dnaB518*::Tn*10*/ *supE42 rpsE*	W1485E derivative; SCLAFANI, WECHSLER and SCHUSTER 1981
RS103	*rpsL179 tyrT66*	SCLAFANI and WECHSLER, submitted
RS181	RS103 *dnaB518*::Tn*10*(P1*bac*)	Transduction of lysogen
CR34	*thyA deoC1 rpsL*	WECHSLER and GROSS 1971
E391	CR34 *dnaB391*	WECHSLER and GROSS 1971
CR63	*supD60 lamB*	APPLEYARD, MACGREGOR and BAIRD 1956; D. PARMA
RS195	594 *srl*	Transduction; *srl* deletion derived from *srlC300*::Tn*10*
JC5519	*recB21 recC22*	*E. coli* Genetic Stock Center
JC5088	HfrKL-16 *recA56*	*E. coli* Genetic Stock Center

phage λ at any temperature (SCLAFANI, WECHSLER and SCHUSTER 1981). As part of the genetic analysis of the *dnaB*::Tn*10* insertions, we attempted to transduce nonlysogenic, *dnaB*+ recipients to Tet^R with a lysate grown on a *dnaB*::Tn*10*, P1*bac* lysogen. If the donor Tn*10* insertion were in *dnaB* and did not transpose to a new location in the recipient, no Tet^R transductants should have been observed, as an unsuppressed *dnaB*::Tn*10* would be lethal. To circumvent the possibility that, if a P1 lysate were made on the *dnaB*::Tn*10* (P1*bac*) donor, many cells might become P1*bac* lysogens and, thus, be suppressed for the *dnaB*::Tn*10* phenotype, T4GT7 was used as transducing phage (WILSON *et al.* 1979). Much to our surprise, Tet^R transductants of the nonlysogens were isolated at 30–60% of the frequencies observed when isogenic P1*bac* lysogens were used as recipients. The Tet^R transductants isolated from nonlysogenic recipients were unstable for Tet^R even after two or three colony purifications and were cryoresistent (Cry+); Tet^R transductants of P1 *bac* lysogens, on the other hand, were stable and Cry$^-$ (data not shown). Even if the nonlysogenic Tet^R transductants were grown in the presence of tetracycline to maintain selection for Tet^R, they were able to support growth of λ bacteriophage. Since λ replication absolutely requires, and strain survival depends on, *dnaB* activity, this observation is indicative of the presence of the *dnaB*+ allele in these Tet^R strains.

These Tet^R *dnaB*+ strains lacking P1*bac* prophage could have arisen in two ways: the Tn *10* could have transposed to a location other than the *dnaB* gene, or the transductants could result from duplication of the region and contain both *dnaB*+ and *dnaB*::Tn*10* alleles. The pronounced instability of the Tet^R phenotype suggested to us that the Tn*10* had not moved to a new site on the chromosome and that either there was a duplication or there had been a transposition of the Tn*10* to an extrachromosomal element. As none of the transductants of interest were Cm^R (a phenotype conferred by the *Cm-1* gene of the phage) or P1 immune, it was concluded that they were not lysogenic for P1 (data not shown). The fact that P1*vir*^{s} lysates made on the Tet^R Cry+ transductant strains under selective conditions (tetracycline present) did not produce high-frequency transducing lysates for Tet^R, but transduced Tet^R at normal frequencies, supported the conclusion that the transductants were not lysogenic for a P1 carrying Tn*10*. Tet^R transductants were not obtained if recombination deficient, *recA* strains, whether lysogenic for P1*bac* or not, were used as recipients (data not shown), thereby providing further evidence against the possibility that an unknown plasmid or a cryptic P1 prophage carried the transposon.

To analyze the putative duplications more carefully, similar transductions to those described above were done using a *dnaB*::Tn*10* *malB*+ *donor* [*malB* is co-transducible with *dnaB* (BACHMANN and LOW 1980)] and a *dnaB*+ *malB* recipient. This region of the chromosome is shown in Figure 1. Co-transduction of Tet^R and MalB+ (selection was for Tet^R) was obtained at normal frequency (~48% and 23% with T4GT7 and P1 transducing phages, respectively), but both phenotypes were unstable. If this observed instability resulted from the presence of duplications, it suggests that such duplications could extend from *dnaB* through the *malB* locus.

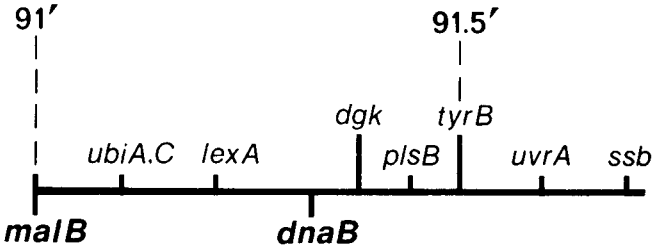


FIGURE 1—Map of the *malB–dnaB* region of the *Escherichia coli* K12 chromosome. The notation of 91′ and 91.5′ designate minutes on the standard chromosome map (BACHMANN and LOW 1980).

To show that the *dnaB* locus was duplicated in Tet^R Cry$^+$ strains, cultures were grown under selective pressure with tetracycline, infected with P1*bac*, and streaked onto plates lacking tetracycline, but containing chloramphenicol (to select for P1*bac* lysogens) at 37°. Results of one of these experiments are shown in Table 2. A substantial fraction of the resultant colonies were Tet^R Cry$^-$ and could not support λ growth; thus, these colonies had the phenotype of a *dnaB*::Tn*10* P1*bac lysogen* (Table 2). If the same culture was infected with P1*bac ban*, which cannot supply *ban* function (D'ARI *et al.* 1975), only Tet^S segregants were found (data not shown). In addition, these Tet^S segregants were nearly always Mal$^-$. These results indicate that the transposon has remained in its original position (*dnaB*::Tn*10*) and that the *dnaB* and *malB* loci are frequently duplicated in the same clone.

The stability of the duplications: In all cases, we have measured duplications following a transduction and have used segregation of the apparently duplicated loci as a major criterion. The structure of the duplicated region can be discerned from an analysis of the segregation pattern. The data in Table 2 show the segregation pattern for one duplication. Of the 100 colonies picked, nine lost the *dnaB*::Tn*10* allele, while 75 lost the *dnaB*$^+$ allele. The *malB*$^+$ gene had a strong

TABLE 2

*Segregation of duplicated markers**

Number	Mal	Phenotype†		λ‡
		Tet	Cry	
16	+	R	+	+
1	+	S	+	+
0	—	R	—	—
74	+	R	—	—
1	—	R	+	+
8	—	S	+	+

* JW165Dp3 (*malB*+ *dnaB518*::Tn*10*) (*malB45 dnaB*+) was infected with P1*bac*; after selection for CM^R, 100 colonies were analyzed. The notation Dp for duplications followed by the number of the duplication and brackets surrounding the duplicated markers is after JOHNSTON and ROTH (1980).

† +, wild type; —, mutant; R, resistant; S, sensitive.

‡ Ability to support growth of λ.

tendency to co-segregate with its original neighboring locus, *dnaB*::Tn*10*, since 74 of 75 Tet^R segregants were Mal$^+$. Similarly, eight of nine Tet^S segregants were Mal$^-$. These results imply that, for this particular duplication, the two loci remained linked as in their original haploid parents. The structure of the duplication must have been ($malB_x$ $dnaB_x$) ($malB_y$ $dnaB_y$) where the subscripts x and y represent the two original parents.

The duplication analyzed in Table 2 segregated haploid types at high frequency. For some other duplication-carrying transductants, however, segregation of haploid types, Tet^S Cry$^+$ or Tet^R Cry$^-$ colonies, did not appear at a high frequency (Table 3). This variation in segregation can be interpreted as indicative of the size of the duplicated regons, *i.e.*, the larger the duplication, the greater the opportunity for recombination between the two copies of the *dnaB* gene by an intrachromosomal event. This has been suggested previously by others (ANDERSON and ROTH 1977, 1978). Though the structure of a duplication can also influence segregation frequency, the simplifying assumption correlating size and segregation frequency appears to be largely valid and will be used exclusively here. In order to provide further evidence for this idea, we made P1*vir*^{s} lysates on some of the nonlysogenic duplicated strains under selective conditions, and, using these lysates as donors, transduced either *malB* (P1*bac*) or *lamB* (P1*bac*) lysogens to Tet^R at 37° (Table 3). Duplications that readily segregated Tet^S clones gave rise to predominantly haploid transductants (Table 3, lines 1 and 3), while one duplication-bearing strain, which segregated more slowly, gave rise to all duplicated transductants (line 2). Linkage of Tn*10* to either *malB* or *lamB* (*malB* and *lamB* are highly, > 95%, co-transducible) was not destroyed even in the case in which all transductants were duplicated. Some of the duplications were unstable for the *lamB* gene (line 2), and a small number segregated Mal$^-$ colonies.

Figure 2 is a schematic diagram showing how duplications may be generated by an unequal crossover event between reiterated homologous sequences. A consequence of the duplication is the formation of the novel joint, *i.e.*, a sequence not found in the haploid genome, *m* next to *f* (HERSHEY 1970; ANDERSON and ROTH

TABLE 3

Genetic analysis of duplications

Donor	Tn*10* segregation*	Recipient marker	Tet^R duplications† / Total Tet^R	LamB$^+$ or MalB$^+$ / Total Tet^R
1. RS121 Dp1	Fast (87%)	*lamB*	4% (4/100)	47%
2. RS121 Dp2	Slow (2%)	*lamB*	100% (100/100)	14%
3. JW165 Dp3	Fast (84%)	*malB*	1.9% (1/54)	11%
4. RS116	None	*lamB*	8% (8/100)	34%
5. RS116	None	*malB*	14% (14/100)	23%

All donors were MalB+ LamB+ Tet^R. Transductions were for Tet^R at 37° into CR63(P1*bac*) for LamB+ and into RS121 (P1*bac*) for MalB+. Transducing lysates were P1*vir*^{s}, except for RS116 in which the phage vector was P1*bac* by induction.

* Cells were streaked onto nonselective medium after growth in tetracycline-containing broth for >10 generations; 100 colonies were picked and the number of Tet^S determined.

† Tet^R duplications measured as Cry+, unstable Tet^R transductants.

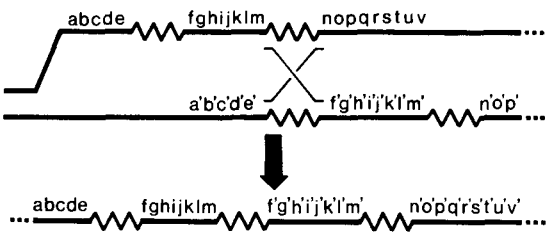


FIGURE 2—Duplication formation. Letters represent sequences, and primes indicate the homologous sequences on the sister duplex of a replicating chromosome. The zigzag segments indicate sequences of repetitive homology, and a crossover at such sequences is shown. A duplication and a deletion result from the recombination event, but only the duplication is depicted.

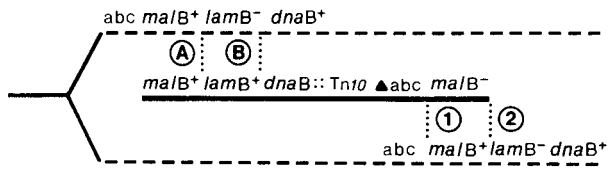
1977). Transduction of the novel joint or of the novel joint plus one segment of the duplication can lead to transduction of the duplication. If the selected marker employed for detecting the duplication were close to the novel joint, the probability of co-transducing the selected marker and the novel joint and, hence, of generating a duplication in the recipient would be very high. In addition, if the duplicated region were small, not only would the selected marker necessarily be close to the novel joint, but also the entire duplication might be transducible intact. With small duplications, the chance of crossovers between the two copies would be relatively low and, consequently, segregation would be rare. Line 2 of Table 3 presents data consistent with this reasoning, as a slow segregator used as a transductional donor results in recombinants all of which carry a duplication of the scored marker. Thus, co-transduction of *dnaB*::Tn*10* and the novel joint is 100%. (These duplication-bearing transductants do segregate haploid types at low frequency.)

In the experiment in Table 3, every cell in the donor population contains a duplication (lines 1–3). A generalized schematic diagram of such an experiment and the resulting expected combinations of recipient and donor alleles are shown in Figure 3. Transduction of the novel joint can result in transductants carrying duplications of *dnaB* and the other co-transducible loci.

If the duplication were large and the selective marker were far from the novel joint, segregation of the duplicated markers would be relatively common, and co-transduction of the selected marker and the novel joint would be infrequent. The fast-segregating duplications of lines 1 and 3 of Table 3 give rise to a relatively low frequency of duplications in transductions and, thus, support the above scheme.

Recombination dependence: It was expected that segregation of duplicated markers would be effected by the normal, legitimate recombination systems. Therefore, we tested for dependence on *recA* and on *recB,C* function in this regard.

A *srl* nonlysogenic recipient was tranduced to Tet^R using *dnaB*::Tn*10* as donor, and transductants were shown to be unstable. Three such colonies were grown under selective conditions and mated with strain JC5088 (Hfr *recA56*), selecting Srl^+ Tet^R exconjugants. These were screened for recombinants carrying *recA* by testing for sensitivity to methyl methanesulfonate (MMS^S) after purification on



CROSSOVER	RESULTANT GENOTYPE
Ⓐ & ①	abc malB+ lamB+ dnaB ::Tn10 ▲abc malB+ lamB− dnaB+
Ⓐ & ②	abc malB+ lamB+dnaB:: Tn10 ▲abc malB− lamB− dnaB+
Ⓑ & ①	abc malB+ lamB− dnaB:: Tn10 ▲abc malB+ lamB− dnaB+
Ⓑ & ②	abc malB+ lamB− dnaB:: Tn10 ▲abc malB− lamB− dnaB+

FIGURE 3—Transduction of duplicated sequences and the novel joint. Dashed and bold solid horizontal lines underscore duplicated sister chromosomes and a transducing fragment, respectively. (▲) represents the position of the novel joint. Dotted vertical lines indicate the location of a genetic crossover. These crossovers are labeled 1, 2, A or B, and the resultant genotypes of combinations of such events are shown in the lower part of the figure.

nutrient media containing tetracycline. All Rec$^+$ clones (50/50) still segregated Tet^S colonies, while *recA* derivatives (50/50) did not. Then, 10 *recA* derivatives were transduced to Rec$^+$ by selecting for MMS^R, and all these were purified on nutrient tetracycline plates. Upon streaking on nonselective media, all Rec$^+$ transductants now yielded Tet^S segregants. These results provide firm evidence that segregation is dependent upon the *recA* gene product, and therefore, apparently on recombination.

On the other hand, the absence of *recB,C* function did not affect segregation. Duplications were isolated in a *thyA* strain, CR34, and were transduced to Thy$^+$ using a P1*vir*^{s} lysate of the *recB,C* strain, JC5519. The *recB,C* marker, defined as MMS^S and sensitive to UV, co-transduced with *thy*$^+$ at 40%. After two colony purifications, these *recB,C* colonies (10/10) yielded Tet^S segregants at a frequency similar to the Rec$^+$ (10/10) transductants.

This lack of apparent involvement of *recB,C* function adds another instance to those already known in which the *recA* and *recB* functions affect recombination differently. In some cases, *recB* strongly influences recombination at an early step and, in others, at a late step in the process (PORTER, MCLAUGHLIN and LOW 1978). Chromosome mobilization by F′ factors in primary zygotes of *recB* or *recC* strains exceeds 50% of wild-type levels, but decreases after establishment of the F′ (WILKINS 1969). It has been suggested that the *recB,C* product, exonuclease V, may play a more important role in the viability of newly formed recombinants than in the actual recombination mechanism (CLARK 1973). With tandem duplications, the situation is different from any of those mentioned above, as both participants in the recombination event are presumably supercoiled. The absence of exonuclease V may not affect such an event or may affect only the deleted portion

of the duplication and, thus, not decrease viability. In any case, segregation of the duplicated markers was not influenced by *recB,C* mutations.

*The duplications are independent of Tn*10: In order to determine whether the transposon itself could be involved in the generation of the duplications, we performed both P1*vir*^{s} and T4GT7 generalized transductions, using a temperature-sensitive *dnaB* recipient (E391) and a *dnaB*$^+$ strain as donor (RS103); selection was for *dnaB*$^+$ (temperature resistance). Colonies selected at 42° were then streaked out at 30° and screened for ts segregants. P1*vir*^{s} and T4GT7 yielded 13% (13/100) and 8.3% (8/96) colonies, respectively, which subsequently segregated ts colonies after two colony purifications (Table 4). Reconstitution experiments showed that no ts colonies could be obtained if *dnaB*$^+$ and *dnaB391* cells were mixed, plated out at the restrictive temperature and the resulting colonies tested for segregation after streaking at 30°. Thus, the segregants are not merely mixtures of temperature-sensitive and temperature-resistant cells. These data show that a region that includes the *dnaB* locus can be duplicated and that formation of these duplications does not require the presence of a Tn*10* insertion.

Frequency of the duplications: In an attempt to quantitate the frequency of duplications existing in a population at a given time, we performed generalized transductions with either T4GT7 or P1*vir*^{s}, using various recipients and/or conditions (Table 4).

Method (1) P1 or T4 lysates made on *dnaB*::Tn*10* strains were used to transduce isogenic P1*bac* lysogenic or nonlysogenic recipients to Tet^R at 37°, and the results of the two transductions were compared. The number of *dnaB*::Tn*10* transductants obtained with the nonlysogens, divided by the number observed with the lysogens, was the frequency of duplication. Method (2) P1*bac* lysogens of various strains were used as recipients and transduced with T4GT7·*dnaB*::Tn*10*. Transductants were plated for Tet^R at 37° to determine the total number of Tet^R transductants and at 25° to determine the number of duplications. Also, Tet^R colonies obtained at 37° were replica-plated and screened for cryoresistant clones. The number of Tet^R transductants at 25°, divided by the number at 37°, gives an estimate of the frequency of duplications. Method (3) A *dnaB*(ts) recipient was transduced to temperature resistance. Colonies were then screened for ability to segregate *dnaB*(ts) clones after two rounds of colony purification at 30°.

These data indicate that the estimated frequency of duplications in the *dnaB malB* region can vary from 6 to 60%, depending on the method used (Table 4). Method 1 consistently gave higher frequencies, while method 2 yielded lower frequencies. Methods 2 and 3 may produce more accurate measurements as the frequency of duplications is estimated, in both of these methods, from the product of a single transduction. On the other hand, the frequencies using method 3 were obtained following two purifications and would be expected to be low since any segregation occurring at earlier stages would not have been scored. Frequencies often vary from culture to culture, even if the donor lysate is the same (Table 4), which implies that the recipient population itself must influence the observed frequency of duplication, *i.e.*, two copies of the loci are present in the recipient, with only one copy receiving the donor DNA. In addition, the region of the chromo-

TABLE 4

Duplication frequencies

Phage vector	Donor	Donor genotype	Recipient	Recipient genotype	Frequency *dnaB*Dp†	Mal+ among *dnaB*Dp	Method‡
T4	RS181	*dnaB*::Tn*10 malB*+	RS121	*dnaB*+ *malB*	60%	60%	1
T4	RS181	*dnaB*::Tn*10 malB*+	JW165	*dnaB*+ *malB*	34%	35%	1
T4	RS181	*dnaB*::Tn*10 malB*+	JW165(P1*bac*)	*dnaB*+ *malB*	6.25%	40%	2
T4	RS181	*dnaB*::Tn*10 malB*+	RS121(P1*bac*)	*dnaB*+ *malB*	8.2%	45%	2
P1	RS113	F′ 118 *dnaB*::Tn*10*/*dnaB*+	594	*dnaB*+	62.5%	—	1
P1	RS103	*dnaB*+	E391	*dnaB*(Ts)	8.3%‡	—	3
T4	RS103	*dnaB*+	E391	*dnaB*(Ts)	13%‡	—	3

† See text.
‡ Frequency determined after two colony purifications.

some influences the frequency since *his* duplications appeared at frequencies of $< 0.1\%$ when a His$^+$ recipient was transduced to Tet^R [His$^+$], using transducing phage grown on a *his*::Tn*10* insertion mutant (method 2). These controls employed the same *dnaB*$^+$ strains used for measuring *dnaB*::Tn*10* duplications.

DISCUSSION

We have described the isolation and partial characterization of tandem duplications that include the *dnaB* locus of *E. coli*. Duplication-carrying transductants were obtained following generalized transductions mediated either by phage P1 or by T4. We believe that these transductants carry chromosomally located tandem duplications because they are unstable and dependent on bacterial recombination functions for segregation. In addition, *dnaB* and adjacent loci can be simultaneously duplicated, and some duplications can be transduced. Duplications in *E. coli* and *S. typhimurium* have been isolated previously (for review see ANDERSON and ROTH 1977), but only in one case, involving the lethal amber suppressor, *trpT* (SOLL and BERG 1969), have duplication frequencies approached those described here. One of our methods for estimating the duplication frequency (method 1) is most similar to that used for *trpT* (SOLL and BERG 1969), while methods 2 and 3 are like those used in Salmonella (ANDERSON and ROTH 1978). Method 1 consistently yielded higher frequencies, perhaps because of the selection scheme employed. Yet, regardless of the method used, duplications in this region are detected at high frequencies. The high frequency of duplications in this region may explain why some dominant *lexA* mutants (*lexA* maps between *malB* and *dnaB*, Figure 1) were difficult to purify (D. MOUNT, personal communication).

The *malB–dnaB* region of the chromosome presumably contains highly preferred duplication-generating sequences. If one assumes that homologous sequences, *e.g.*, IS-like sequences (CALOS and MILLER 1980), are scattered about the region, unequal sister chromosome exchange could create duplications by either legitimate or illegitimate recombination mechanisms (FRANKLIN 1971; Figure 2). As diagrammed in Figure 2, if the *dnaB* locus is represented by the sequence *jk* and *malB* by *mn*, then although *malB* has been split, one functional *malB* exists, and can be co-transduced with *dnaB*. In addition, segregation of the duplicated *malB*, in this case, would yield different *malB* types only if the mutated sequence were located in *m*, as *n* is no longer present on the chromosome; RS121Dp2 (line 2, Table 3) may be of this type. Duplications segregating at high frequencies (lines 1and 3, Table 3) could rely upon other sequences for their formation. It has been suggested that homologous ribosomal RNA genes (*rrn*), which are found between 70 and 90 minutes on the *E. coli* genetic map (BACHMANN and LOW 1980), may be responsible for duplications in this region (ANDERSON and ROTH 1978), as had been shown for the *glyT* locus (HILL *et al.* 1977). However, the *dnaB* region at 91.3 minutes is not between these *rrn* cistrons, so that either other unidentified *rrn* sequences are present clockwise from *rrnE* (90 min) or other different sequences are responsible. The *glyS* region is known to form duplications, and it is also not flanked by *rrn* sequences (CAPAGE and HILL 1979).

Our experiments were concerned with showing that duplications occurred at high frequency in the region surrounding *dnaB*. No attempt was made to investigate the mechanism by which these duplications are generated. However, the segregation frequency as measured in whole cultures of isolated duplication strains was always a few percent and, thus, in genetic terms, very high. This suggests that duplication and subsequent reduction constantly occurs. Segregation of duplicated markers is dependent on *recA* function, but not on *recB,C* product.

The experiments of Table 3, which are diagrammed in Figure 3, show that pre-existing duplications can be transduced from the donor to the recipient cell. In all cases, however, the duplications used were obtained after transduction. Two observations made in the course of these experiments suggest that the isolation of duplications in this manner is usually dependent on pre-existing duplications in the recipient. Such duplications would become apparent only when donor material recombined into one of the duplicated copies in the recipient. The observed variation in the frequency of duplications when one donor lysate was used with different recipients (Table 4) supports this idea. Additional evidence that this is true was obtained when BD1154 (WARNER and ROCKSTROH 1980) was used as recipient. This was the only *E. coli* strain tested in which duplications of the *dnaB* region were obtained at low frequency (~0.3%). If reiterated sequences of different types are responsible for the creation of duplications, some strains may have fewer of these homologous sequences and, therefore, be less likely to form tandem duplications. Strain BD1154 was constructed by an Hfr cross in which much of the donor chromosome was transferred to the zygote, and we have not attempted to determine the genesis of its low propensity to form duplications in the *dnaB* region. The high frequencies of duplication in the *malB–dnaB* region, as reported in this paper, have been observed in the following well-known K12 backgrounds: CR34, W3110, 594 and AB1157 (BACHMANN 1972).

We can speculate that *dnaB* and/or other neighboring genes may be duplicated during DNA replication by recombination between the replicating sister chromosomes. Duplications may reflect the existence of a gene amplification control mechanism. Since haploid types would segregate spontaneously if environmental conditions were to change, such a mechanism has great flexibility and would, presumably, provide a selective advantage. We were not able to decrease the duplication frequency by growing cells in glucose minimal media or even in acetate minimal medium in which cells are expected to contain only one nuclear region (LARK 1966); thus, duplication, if it occurs by a recombination mechanism of the type described above, is generated from within one nuclear region and not by recombination between chromosomes separated into different regions.

We are especially grateful to JOHN ROTH for critical comments on the manuscript and to FOREST CHUMLEY for helpful suggestions in the early stages of this work. We thank GORDON LARK and C. I. DAVERN for their hospitality.

These experiments were conducted by R. A. SCLAFANI in partial fulfillment of the Ph.D. requirements at Columbia University.

This work was supported by Public Health Research Grant GM-25476.

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Corresponding editor: G. MOSIG