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# Genetic and Segregation Analysis of *Escherichia coli* Strains Containing a Tandem Duplication of the *trpD-purB* Region of the Chromosome

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Genetic and segregation analysis of *Escherichia coli* strains containing a partial duplication of the *trp* operon reveal that the 2.5-min-long region *trpD-purB* is duplicated in tandem in the chromosome. The adjacent loci *cysB* and *fabD* are not duplicated. Although one copy of the duplicated region is longer than the maximum size of bacteriophage P1*kc* transducing fragments, the frequency at which the duplicated segment *trpDCBA* is transferred by transduction to *tonB-trp* deletion strains is equal to that observed for transfer of the normal *trp* operon. This suggests that three-point recombination events believed to account for transduction of long duplications occur as frequently as two-point recombination events believed to account for normal transduction. Cotransduction frequencies of *trpDCBA* with the duplicated loci *tonB, galU, tyrT,* and *hemA* are very similar to those for the *trp* operon with the same loci. This indicates that normal genetic linkage is maintained during the three-point recombination event. However, *purB,* which is normally unlinked to *trp* by transduction, is closely linked to *trpDCBA* and thus must be near the repeat point of the duplication. Transduction tests with point mutations in the *trp* operon indicated that the repeat point occurs near the normal boundary between *trpE* and *trpD*. Segregation analysis of heterogenotes constructed from *tonB-trp* deletion strains shows that the frequency at which a marker is lost is approximately proportional to its distance from the repeat point. This finding is consistent with a random, single-site crossover event during segregation. Several observations indicate that nonreciprocal genetic exchange also occurs between copies of the duplication. Analysis of heterogenotes containing *dadR1* and *dadR*$^+$ demonstrate that the mutant allele is transdominant.

Genetic duplications have been reported for several regions of the bacterial chromosome in *Escherichia coli* (4, 6-8, 10-14, 22, 24-26) and *Salmonella typhimurium* (1, 2, 19, 21, 27, 28). In most cases, the duplicated segments are believed to be arranged in tandem, although several examples of translocation (insertion) duplications have been reported (4, 10, 14), and in one case the duplicated segment is carried by a plasmid (1, 19). The size of duplications varies from as little as one known gene (8, 22) to approximately one-third the bacterial chromosome (26). In many cases, however, sufficient genetic information is not available to determine the exact size and position (tandem or translocated) of the duplications. One feature common to all duplications is instability. When grown under nonrestrictive conditions, bacteria lose duplications at relatively high frequencies (0.1 to 50% of colonies tested) by a recombination (*recA*)-dependent process (4, 7, 11, 24, 28). For tandem duplications, the loss of material can best be explained by a single-site recombination event between the duplicated segments followed by segregation of the chromosome lacking the duplication (11, 12). However, the fate of the material that is lost from the chromosome has not been determined. The mechanism(s) by which duplications are formed is poorly understood. In one case (28) it is reported to be dependent on the *recA* system, whereas in another case (4) it is not. UV light (10) and chemical mutagens (10, 26) stimulate the frequency of occurrence. Thus, both pyrimidine dimer formation and specific base changes in DNA may be involved.

Previously we reported a duplication containing the operator-distal genes *D-C-B-A* of the *trp* operon linked to a transcription promoter which is not subject to tryptophan repression (24). The

duplication was obtained in a mutant strain RM213 selected for resistance to anthranilic acid. Growth of the parental strain RM106 containing two strongly polar, operator-proximal *trpE* mutations [*trpE9829*(Am) *trpE9851*(Oc)] is very sensitive to high concentrations (30 μg/ml) of anthranilic acid. Sensitivity of strain RM106 results from anthranilic acid inhibition of the *trpD* and *trpC* polypeptides which are markedly diminished by polar *trpE* mutations (32). Thus, mutations that relieve or obviate the polar effect on *trpD* and *trpC* result in resistance to anthranilic acid. Among 18 independently isolated anthranilic acid-resistant mutants of strain RM016, only strain RM213 was found to contain a duplication (M. H. Simonian and R. D. Mosteller, unpublished observations). Previously we showed that streptomycin resistance (*strA*) mutations increase the loss of the duplication 5- to 10-fold (24). The reason why these mutations, which alter ribosomal protein S12 (3), affect loss of the duplication is not known.

In this paper, we describe a more thorough genetic analysis of the duplicated region in strain RM213 and present segregation analysis of heterogenotes containing the duplication. In the latter studies, the frequency at which a particular genetic locus is lost is related to its position in the duplication. In addition, these studies were used to demonstrate that the mutant allele *dadR1*, which enables some amino acid auxotrophs to utilize D-amino acids in place of the respective L-amino acids (17), is transdominant to the wild-type allele *dadR*$^+$. Using an analogous selection procedure, Jackson and Yanofsky (14) obtained several duplications of the *trp* operon which are translocated rather than tandem. Their studies did not include determination of the size or exact location of these duplications.

## MATERIALS AND METHODS

**Bacterial strains, bacteriophage, and techniques.** All bacterial strains are derivatives of *E. coli* K-12 except strains KB30 and MS445, which are hybrids of *E. coli* K-12 and *E. coli* B (Table 1). The *trpE* and *trpD* mutants used for mapping (see Table 7) and bacteriophages T1, T4*amE*4306, φ80*vir*, and φ80h$^-i^\lambda$ were obtained from C. Yanofsky. Transductions were performed with bacteriophage P1*kc* (18). For spot transductions, a drop of P1*kc* lysate was placed on a lawn of recipient cells ($2 \times 10^8$ cells/plate) on tris(hydroxymethyl)aminomethane (Tris)-minimal agar medium (9) supplemented with 0.2% glucose, 0.05% acid casein hydrolysate, and 2.5 mM $CaCl_2$. Growth was usually scored after incubation for 24 h. F$'$ × F$^-$ conjugations were performed by mixing equal volumes of exponentially growing cultures ($2 \times 10^8$ cells/ml) in L-broth medium, incubating for 1 h, and then spreading on selective medium. Hfr × F$^-$ conjugations were performed similarly, except that 10-fold fewer donor cells were used and incubation was for 2 h. Colicin V,B was harvested from a mitomycin C-induced culture of strain YS57 obtained from C. Yanofsky.

**Growth medium and conditions.** All incubations were at 37°C, except that strains containing the temperature-sensitive episome F42-114 or the *fabD1* allele were incubated at 30°C. Cultures were incubated with shaking in minimal medium E (29) supplemented with 0.2% glucose or in L-broth medium (1% tryptone [Difco], 0.5% yeast extract [Difco], 0.5% NaCl, 0.1% glucose). Solid medium contained 1.5% (minimal or Tris-minimal) or 1.3% agar (L broth). Supplements were added when needed as follows: 20 μg of L- or D-trytophan, indole, L-threonine, or Δ-aminolevulinic acid per ml, 30 μg of anthranilic acid per ml, 100 μg of L-cysteine hydrochloride per ml, 50 μg of L-glutamic acid or thymine per ml, and 10 μg of adenine per ml.

**Selection and phenotypic characterization of strains.** All *trpB*$^+$ strains, including *trpE* mutants, *tonB-trpA* deletion mutants, and *trpDCBA*-containing strains, can utilize indole in place of tryptophan. In addition, *trpDCBA*-containing strains but not *trpE9829 trpE9851* mutants can utilize 30 μg of anthranilic acid per ml in place of tryptophan. The *trpB* mutants and *tonB-trpAE* deletion mutants can utilize tryptophan only.

TonB$^-$ strains were selected for resistance to colicin V,B and bacteriophage φ80*vir* on L-broth agar medium (5). In some cases TonB$^-$ strains were tested for resistance to bacteriophage T1. TonB$^+$ strains were selected for chromium resistance (30) on unextracted Waring-Werkman (31) minimal agar medium [4 g of $K_2HPO_4$, 1 g of $KH_2PO_4$, 1 g of $(NH_4)_2SO_4$, and 0.1 g of $MgSO_4 \cdot 7H_2O$ per liter) containing 2% agar, 0.2% glucose, and $5 \times 10^{-5}$ M $CrCl_3$. The presence of *att80*$^+$ was determined by testing for stable bacteriophage φ80h$^-i^\lambda$ lysogens. Turbid centers of φ80h$^-i^\lambda$ plaques were cultured in 5 ml of L-broth medium and spread on L-broth agar medium, and the resulting colonies were replica plated onto a lawn of strain RM213. After incubating for 16 h, a large fraction (80 to 95%) of colonies from *att80*$^+$ strains result in lysis spots in the background of bacterial growth. The Gal phenotype of strains was tested on minimal agar medium supplemented with 0.2% galactose in place of glucose. The TyrT3 (suppressor) phenotype was examined by spotting bacteriophage T4 amber mutant E4306 on a streak of bacteria on L-broth agar medium. Suppression was indicated by lysis after incubation for 16 h. The Hem phenotype was usually tested on L-broth agar medium with and without Δ-aminolevulinic acid. The DadR$^-$ phenotype was demonstrated by growth on minimal agar medium supplemented with D-tryptophan in place of L-tryptophan. FabD mutants grow at 30°C but not at 42°C on L-broth agar medium.

## RESULTS

**Chromosomal location of *trpDCBA*.** Preliminary experiments indicate that the duplicated segment of the *trp* operon, *trpDCBA*, can be transferred by bacteriophage P1*kc*-mediated transduction to strains containing either a mu-

TABLE 1. *Bacterial strains*

| Strain | Sex | Genotype[a] | Source/comments[b] |
|---|---|---|---|
| CA10 | HfrH | *galU95 relA1* $\lambda^-$ | S. Brenner strain (CGSC 4973) |
| EC-0 | F42-114 *lac*$^+$ | *thi-1 relA1*? $\lambda^-$ DE5 *supE44*? | J. Beckwith; DE5 is the deletion $\Delta(proB\text{-}lac)_{X111}$ of Jacob; F42-114 *lac*$^+$ is replication defective at 42°C |
| GY854 | F42 *lac*$^+$ | *thr-1 leu-6 thi-1 thyA6 deoC1 pyrF32 uvrB501 lacY1 malA19 codA1 tonA21* $\lambda^r$ $\lambda^-$ *supE44* | R. Devoret strain (CGSC 3078) via R. C. Deonier |
| H680 | F$^-$ | *purB51 thi-1 tyrA2 his-68 trp-45 lacY1 gal-6 mtl-2 xyl-7 malA1 str-125 tonA2 tsx-70 supE44* $\lambda^r$ $\lambda^-$ | P. G. de Haan strain (CGSC 5038) |
| KB30 | F$^-$ | $\Delta$(*att80-tonB-trpAE)2* | C. Yanofsky |
| L48 | F$^-$ | *fabD1 thi-1 gltA5 ara-14 lacY1 galK2 xyl-5 mtl-1 tfr-5 tsx-57 str-20* $\lambda^+$ | D. F. Silbert (23) Formerly designated LA2-89 |
| MS298 | F$^-$ | *trpR2 tna-2* | P1(RM234) × RM256 → Trp$^+$ |
| MS304 | F$^-$ | *trpR2 tna-2* $\Delta$(*tonB-trpAE)1:trpDCBA* | P1(RM213) × RM256 → TrpB$^+$ (anthranilic acid resistant) |
| MS328 | F$^-$ | *thyA110 trpE9829 trpE9851:trpDCBA* | Spontaneous Thy$^-$ of RM213 |
| MS330 | F$^-$ | *strA202 trpE9829 trpE9851:trpDCBA* | Spontaneous Str$^r$ of RM213 (24) |
| MS397 | F$^-$ | *tna-2* $\Delta$(*tonB-trpAE)2* | P1(RM390) × RM310 → Cys$^+$ (Trp$^-$) |
| MS436 | F$^-$ | *tna-2* $\Delta$(*tonB-trpAE)2 thr thyA* | P1(RM390) × RM337 → Cys$^+$ (Trp$^-$) |
| MS445 | F$^-$ | $\Delta$(*att80-tonB-trpAE)2:trpDCBA* | P1(MS304) × KB30 → TrpB$^+$ (anthranilic acid resistant) |
| MS573 | F$^-$ | *strA202 tna-2* $\Delta$(*trpEB)9* | P1(RM417) × RM486 → Str$^r$ |
| MS582 | F$^-$ | DE48 *strA202 trpE9829 trpE9851:trpDCBA* | P1(MS304) × RM342 → anthranilic acid resistant |
| MS583 | F42-114 *lac*$^+$ | DE48 *strA202 trpE9829 trpE9851:trpDCBA* | P1(MS304) × RM355 → anthranilic acid resistant |
| MS630 | F42 *lac*$^+$ | DE48 *strA202 trpE9829 trpE9851:trpDCBA* | GY854 × MS582 → Lac$^+$ (Leu$^+$) |
| RM43 | F$^-$ | *cysB* $\Delta$(*tonB-trpAE)12* | C. Yanofsky |
| RM106 | F$^-$ | *trpE9829*(Am) *trpE9851*(Oc) | C. Yanofsky |
| RM159 | F$^-$ | *tna-2 trpB9578 strA* | C. Yanofsky |
| RM213 | F$^-$ | *trpE9829 trpE9851:trpDCBA* | Spontaneous anthranilic acid-resistant mutant of RM106 (24; this paper) |
| RM234 | F$^-$ | *tna-2* | C. Yanofsky |
| RM256 | F$^-$ | *trpR2 tna-2* $\Delta$(*tonB-trpAE)1* | C. Yanofsky |
| RM296 | F$^-$ | *tna-2 cysB thr* | C. Yanofsky |
| RM309 | F$^-$ | *trpR2 tna-2 cysB* | P1(MS298) × RM296 → Thr$^+$ (5-methyltryptophan resistant) |
| RM310 | F$^-$ | *tna-2 cysB* | P1(MS298) × RM296 → Thr$^+$ (5-methyltryptophan sensitive) |
| RM312 | F$^-$ | *trpR2 tna-2* | P1(RM159) × RM309 → Cys$^+$ (Trp$^+$) |
| RM337 | F$^-$ | *tna-2 cysB thr thyA* | Spontaneous Thy$^-$ of RM296 |
| RM342 | F$^-$ | DE48 *strA202 trpE9829 trpE9851* | UV-induced *proB-lac* deletion (DE48) mutant of MS330; *trpDCBA* lost during selection |
| RM355 | F42-114 *lac*$^+$ | DE48 *strA202 trpE9829 trpE9851* | EC-0 × RM342 → Lac$^+$ (Str$^r$) at 30°C |
| RM390 | F$^-$ | *trpR2 tna-2* $\Delta$(*tonB-trpAE)2* | C. Yanofsky |
| RM417 | HfrR10(*lac*$^+$) | DE48 *strA202 trpE9829 trpE9851* | Spontaneous Hfr of RM355, selected for Lac$^+$ at 43°C; transfers chromosome clockwise starting near *thyA* |
| RM486 | F$^-$ | *tna-2* $\Delta$(*trpEB)9* | C. Yanofsky |
| RM594 | F$^-$ | $\Delta$(*tonB-trpAE)1 tyrT3* ($su_{III}$) | C. Yanofsky |
| RM621-3 | F$^-$ | $\Delta$(*tonB-trpAE)1 tyrT3:trpDCBA tyrT3* | P1(MS304) × RM594 → TrpB$^+$ (TyrT3); does not segregate TyrT$^+$ clones |

TABLE 1—*Continued*

| Strain | Sex | Genotype[a] | Source/comments[b] |
|---|---|---|---|
| RM621-5 | F⁻ | *Δ(tonB-trpAE)1 tyrT3:trpDCBA tyrT⁺* | P1(MS304) × RM594 → TrpB⁺ (TyrT3); segregates TyrT⁺ clones (Table 6) |
| RM621-7 | F⁻ | *Δ(tonB-trpAE)1 tyrT⁺:trpDCBA tyrT3* | P1(MS304) × RM594 → TrpB⁺ (TyrT3); segregates TyrT⁺ clones (Table 6) |
| RM627 | F⁻ | *cysB Δ(tonB-trpA)50* | C. Yanofsky |
| RM680 | F⁻ | *trpR2 tna-2 cysB Δ(tonB-trpA)50* | P1(RM627) × RM390 → TrpB⁺ (Cys⁻) |
| RM755 | F⁻ | *trpR2 tna-2 cysB hemA8* | P1(SHSP19) × RM680 → Trp⁺ (Hem⁻) |
| RM757 | F⁻ | *trpR2 tna-2 Δ(tonB-trpAE)2 hemA8* | P1(RM390) × RM755 → Cys⁺ (Trp⁻) P1 lysogen |
| RM805 | F⁻ | *trpR2 tna-2 Δ(trpLD)102* | Derived in this laboratory; deletion *Δ(trpLD)102* obtained from C. Yanofsky (15) |
| RM880 | F⁻ | *trpR2 tna-2 Δ(tonB-trpAE)2 purB51* | P1(H680) × RM757 → Hem⁺ (Pur⁻) |
| RM881 | F⁻ | *trpR2 tna-2 Δ(tonB-trpAE)2 dadR1* | P1(T3D) × RM757 → Hem⁺ (DadR⁻) |
| RM882 | F⁻ | *trpR2 tna-2 Δ(trpLD)102 tonB3* | Spontaneous TonB⁻ mutant of RM805 selected for resistance to colicin V,B and *φ80vir* |
| RM958 | F⁻ | *Δ(tonB-trpAE)2 fabD1 gltA5 ara-14 lacY1 galK2 xyl-5 mtl-1 tfr-5 tsx-57 str-20 thi-1 λ⁺* | P1(RM390) × L48 → TonB⁻ (Trp⁻); selected for resistance to colicin V,B and *φ80vir* |
| RM960 | F⁻ | *trpR2 tna-2 Δ(tonB-trpAE)2 hemA8:trpDCBA hemA8* | P1(MS304) × RM757 → TrpB⁺ (Hem⁻) |
| RM961 | F⁻ | *trpR2 tna-2 Δ(tonB-trpAE)2 purB⁺:trpDCBA purB51* | P1(MS304) × RM880 → TrpB⁺ (Pur⁺); segregates Pur⁻ clones (Table 6) |
| RM965 | F⁻ | *trpR2 tna-2 Δ(tonB-trpAE)2 dadR1:trpDCBA dadR⁺* | P1(MS304) × RM881 → TrpB⁺ (DadR⁻); segregates DadR⁺ clones (Table 6) |
| RM1052 | F⁻ | *trpR2 tna-2 Δ(tonB-trpAE)2 galU95* | CA10 × RM757 → TonB⁻ Hem⁺ (Gal⁻); selected for resistance to colicin V,B and *φ80vir* |
| RM1053-3 | F⁻ | *trpR2 tna-2 Δ(tonB-trpAE)2 galU⁺:trpDCBA galU95* | P1(MS304) × RM1052 → TrpB⁺ (Gal⁺); segregates Gal⁻ clones (Table 6) |
| RM1053-4 | F⁻ | *trpR2 tna-2 Δ(tonB-trpAE)2 galU95:trpDCBA galU⁺* | P1(MS304) × RM1052 → TrpB⁺ (Gal⁺); segregates Gal⁻ clones (Table 6) |
| RM1088 | F⁻ | *trpR2 tna-2 Δ(tonB-trpAE)2 hemA⁺:trpDCBA hemA8* | P1(RM960) × RM390 → TrpB⁺ (Hem⁺); segregates Hem⁻ clones |
| RM1099 | F⁻ | *trpR2 tna-2 Δ(tonB-trpAE)2 hemA8* | Hem⁻ segregant of RM1088 |
| RM1100 | F⁻ | *trpR2 tna-2 Δ(tonB-trpAE)2 hemA8:trpDCBA hemA⁺* | P1(MS304) × RM1099 → TrpB⁺ (Hem⁺); segregates Hem⁻ clones |
| SHSP19 | F⁻ | *hemA8 metB1 lacY1 str-134 malA1* | A. Săsărman strain (CGSC 4679) |
| T3D | F⁻ | *trpE(T3) dadR1* | J. Kuhn (17) |

[a] All allele designations are those assigned by the CGSC (*E. coli* Genetic Stock Center, Department of Human Genetics, Yale University School of Medicine, New Haven, Conn.) except for the *tna* and *trp* alleles of C. Yanofsky. The *strA202* allele was formerly designated *strA1* (24).

[b] Strains obtained through CGSC where indicated.

tant *trp* operon (e.g., *trpE9829 trpE9851*) or a deletion of the *tonB-trp* region of the chromosome. Thus, *trpDCBA* was transferred by transduction to an Hfr strain (RM417) containing *trpE9829 trpE9851*. Several independent transductants were tested for the ability to transfer *trpDCBA* or *trpE9829 trpE9851* by conjugation to an F⁻ recipient strain MS436 containing a *tonB-trpAE* deletion. Among 707 *trpB*⁺ recombinants examined, 269 contained *trpE9829 trpE9851* (anthranilate-sensitive recombinants) and 439 contained *trpDCBA* (anthranilate-resistant recombinants). Eighteen of the *trpDCBA*-containing recombinants were tested for loss of *trpDCBA*. Four of the recombinants reverted to the phenotype of the *tonB-trpAE* dele-

tion strain used as recipient, and 14 reverted to the phenotype of *trpE9829 trpE9851* strains. These results indicate that *trpDCBA* is an integral part of the chromosome and that it is close to *trpE9829 trpE9851*.

Similar experiments using the $F^-$ strain MS328 (*trpE9829 trpE9851:trpDCBA*) as potential donor and the *tonB-trpAE* deletion strain RM256 as recipient failed to detect transfer of *trpDCBA* or *trpE9829 trpE9851* (less than one recombinant per $10^9$ recipient cells). The same results were obtained using the F42-114 strain MS583 or the F42 strain MS630 as potential donor and strain MS573 [Δ(*trpEB*)*9*] as recipient. We conclude that *trpDCBA* is not part of a transmissible plasmid.

**Cotransduction of *trpDCBA* with *tonB* and *att80* but not *trp* or *cysB*.** To test for genetic linkage of *trpDCBA* with other chromosomal markers, attempts were made to cotransduce *trpDCBA* with the *trp* operon located in its normal site or with other markers (*cysB*, *tonB*, and *att80*) that are located close to the normal site of *trp*. When strain RM213 (*trpE9829 trpE9851:trpDCBA*) was used as donor and the *tonB-trpAE* deletion strains RM256 and MS397 were used as recipients (experiments 1 and 2, Table 2), both anthranilic acid-resistant (*trpDCBA*) and anthranilic acid-sensitive (*trpE9829 trpE9851*) transductants were obtained. Thirteen of the anthranilic acid-resistant transductants were tested for segregants that had lost *trpDCBA*. All segregants from each strain exhibited the phenotype of the *tonB-trpAE* deletion strains used as recipients, thus indicating that *trpDCBA* is not readily cotransducible with the *trp* operon (*trpE9829 trp9851*). The presence of the *tonB-trpAE* deletion was confirmed by genetic tests (spot transductions), using at least one segregant from each of the 13 transductants. It is interesting to note that approximately equal numbers of anthranilic acid-sensitive and -resistant transductants were obtained in experiments 1 and 2 (Table 2). This indicates that *trpDCBA* can be transferred by transduction as readily as the normal *tonB-trpAE* region of the chromosome. When the anthranilic acid-sensitive parental strain RM106 is used as donor (experiment 3, Table 2), only anthranilic acid-sensitive transductants are obtained. Thus, *trpDCBA* or similar duplications are not generated during transduction. One of the anthranilic acid-resistant strains, designated strain MS304, from experiment 1 (Table 2) was used as a source of *trpDCBA* in further studies. As shown in experiment 4 (Table 2), only anthranilic acid-resistant transductants are obtained when strain MS304 is used as donor.

The presence of $tonB^+$ was examined in strain MS304 by testing for sensitivity to bacteriophage T1. The results indicate (Table 3) that $tonB^+$ was transferred with *trpDCBA* during transduction. Similarly, when *trpDCBA* was transferred by transduction to strain KB30 containing a deletion of the *att80-tonB-trpAE* region, both $tonB^+$ and $att80^+$ were also transferred (Table 3, strain MS445). Since *tonB* and *att80* are normally closely linked to *trp* (but *trpDCBA* is not), these data suggest that *tonB* and *att80* are duplicated in the parental strain RM213.

Attempts to cotransduce *trpDCBA* and *cysB* using either strain RM213 or strain MS304 as donor were not successful (experiments 1 and

TABLE 3. *Tests for tonB and att80 in trpDCBA-containing transductants*[a]

| Strain | Relevant genotype | Phenotype | |
|---|---|---|---|
| | | TonB | Att80 |
| RM106 | *trpE9829 trpE9851* | + | NT |
| RM256 | Δ(*tonB-trpAE*)*1* | − | + |
| MS304 | Δ(*tonB-trpAE*)*1*: *trpDCBA* | + | + |
| KB30 | Δ(*att80-tonB-trpAE*)*2* | − | − |
| MS445 | Δ(*att80-tonB-trpAE*)*2*: *trpDCBA* | + | + |

[a] Strains MS304 and MS445 were constructed by transduction of strains RM256 and KB30, respectively (Table 1). NT, Not tested.

TABLE 2. *Transfer of the trp operon or trpDCBA by transduction*

| Expt no. | Strain and relevant genotype | | No. of $TrpB^+$ transductants[a] | |
|---|---|---|---|---|
| | Donor | Recipient | Anthranilic acid sensitive (*trpE9829 trpE9851*) | Anthranilic acid resistant (*trpDCBA*) |
| 1 | RM213 *trpE9829 trpE9851:trpDCBA* | RM256 Δ(*tonB-trpAE*)*1* | 243 | 255 |
| 2 | RM213 *trpE9829 trpE9851:trpDCBA* | MS397 Δ(*tonB-trpAE*)*2* | 97 | 103 |
| 3 | RM106 *trpE9829 trpE9851* | RM256 Δ(*tonB-trpAE*)*1* | 220 | 0 |
| 4 | MS304 Δ(*tonB-trpAE*)*1:trpDCBA* | RM256 Δ(*tonB-trpAE*)*1* | 0 | 200 |

[a] $TrpB^+$ transductants selected on indole-supplemented medium and tested for anthranilic acid resistance by replica plating.

2, Table 4), although normal genetic linkage of *trp* and *cysB* was observed (experiment 3, Table 4). These findings indicate that *cysB* is not linked to *trpDCBA* and is probably not duplicated.

**Cotransduction of *trpDCBA* with markers in the *tonB-purB* region.** The data in Table 3 suggest that *trpDCBA* is linked genetically to markers located counterclockwise from *trp* on the *E. coli* K-12 genetic map (3). Therefore, we tested for cotransduction of *trpDCBA* with several loci in this region, including *tonB, galU, tyrT* (formerly designated *supF* or $su_{III}$), *hemA, dadR, purB,* and *fabD* (experiments 1 through 7, Table 5). The cotransduction frequency of *trpDCBA* with each marker, except *purB,* is similar to that observed with the *trp* operon in its normal position (experiments 8 through 14, Table 5). The *purB* marker is readily cotransducible with *trpDCBA* (experiment 6, Table 5) but not with the *trp* operon (experiment 14, Table 5; reference 17). The *fabD* locus is not cotransducible with *trpDCBA* (experiment 7, Table 5) or the *trp* operon (23). Several control experiments were performed to test the genetic linkage of *hemA, dadR,* and *purB* (experiments 15 through 17, Table 5). The results differed only slightly from that expected from the *E. coli* K-12 genetic map (3). Our findings are consistent with (i) a duplication of *trpDCBA* through *hemA* (or *dadR*) that is translocated to a site between *purB* and *fabD,* or (ii) a duplication of *trpDCBA* through *purB* that is tandem to the normal *trp-purB* region. The segregation analysis of heterogenotes presented below supports the latter possibility.

**Segregation of genetic markers in heterogenotes.** We have found that all segregants

TABLE 4. *Non-cotransducibility of cysB and trpDCBA*

| Expt no. | Strain and relevant genotype | | Selected marked | No. of transductants[a] | |
|---|---|---|---|---|---|
| | Donor | Recipient | | Total | *cysB*$^+$ |
| 1 | RM213 *trpE9829 trpE9851:trpDCBA* | RM43 *cysB Δ(tonB-trpAE)12* | *trpDCBA* | 738 | 0 |
| 2 | MS304 *Δ(tonB-trpAE)1:trpDCBA* | RM43 *cysB Δ(tonB-trpAE)12* | *trpDCBA* | 696 | 0 |
| 3 | RM106 *trpE9829 trpE9851* | RM43 *cysB Δ(tonB-trpAE)12* | *trpB*$^+$ | 1,110 | 450 |

[a] Total transductants were selected on L-cysteine-supplemented media containing anthranilic acid (experiments 1 and 2) or indole (experiment 3). Equal portions of the transduction mixtures were spread on the same type of media lacking L-cysteine to select for *cysB*$^+$ transductants. Approximately one-third of the transductants obtained in experiments 1 and 2 were also tested by replica plating to medium lacking L-cysteine. All of those tested were Cys$^-$.

TABLE 5. *Cotransduction of trpDCBA or the trp operon with other genetic markers*

| Expt no. | Donor strain | Recipient strain | Selected marker[a] | Unselected marker | Percent cotransduction[b] |
|---|---|---|---|---|---|
| 1 | MS304 | RM882 | *trpDCBA* | *tonB*$^+$ | 58 (42/72) |
| 2 | MS304 | RM1052 | *trpDCBA* | *galU*$^+$ | 57 (124/216) |
| 3 | RM621-3 | MS397 | *trpDCBA* | *tyrT3* | 62 (80/130) |
| 4 | MS304 | RM757 | *trpDCBA* | *hemA*$^+$ | 26 (25/98) |
| 5 | MS304 | RM881 | *trpDCBA* | *dadR*$^+$ | 9 (4/47)[c] |
| 6 | MS304 | RM880 | *trpDCBA* | *purB*$^+$ | 36 (52/144) |
| 7 | MS304 | RM958 | *trpDCBA* | *fabD*$^+$ | 0 (0/78) |
| 8 | RM312 | RM882 | *trp*$^+$ | *tonB*$^+$ | 64 (46/72) |
| 9 | RM312 | RM1052 | *trp*$^+$ | *galU*$^+$ | 68 (146/216) |
| 10 | RM106 | RM594 | *trpB*$^+$ | *tyrT*$^+$ | 43 (55/127) |
| 11 | RM312 | RM594 | *trp*$^+$ | *tyrT*$^+$ | 47 (15/32) |
| 12 | RM312 | RM757 | *trp*$^+$ | *hemA*$^+$ | 32 (57/176) |
| 13 | RM106 | RM881 | *trpB*$^+$ | *dadR*$^+$ | 2 (2/104) |
| 14 | RM312 | RM880 | *trp*$^+$ | *purB*$^+$ | 0 (0/124) |
| 15 | T3D | RM757 | *hemA*$^+$ | *dadR1* | 19 (27/144) |
| 16 | H680 | RM757 | *hemA*$^+$ | *purB51* | 3 (2/72) |
| 17 | T3D | RM880 | *purB*$^+$ | *dadR1* | 39 (28/72) |

[a] The *trpDCBA*-containing transductants of experiment 1 and all *trp*$^+$ transductants were selected on tryptophan-free medium. Other *trpDCBA*-containing transductants and *trpB*$^+$ transductants were selected on indole-supplemented medium. Δ-Aminolevulinic acid was added to the medium when *hemA*$^+$ was the unselected marker, and adenine was added when *purB*$^+$ or *purB51* was the unselected marker.

[b] Transductants with unselected marker/total transductants tested is given in parentheses.

[c] Determined as described in the text.

of strains containing *tonB-trp* deletions (e.g., strain MS304) lose *trpDCBA* and *tonB*$^+$ simultaneously and thus are phenotypically TonB$^-$. This demonstrates that such strains are heterogenotes for the *tonB* locus. In addition, this suggests a convenient method for selecting large numbers of segregants from *tonB-trp* deletion strains containing *trpDCBA*, since the TonB$^-$ phenotype can be selected directly (resistance to colicin V,B and bacteriophage $\phi$80*vir*). Using this procedure, transductants containing the transdominant markers *galU*$^+$, *tyrT3*, *hemA*$^+$, *dadR1*, and *purB*$^+$ (experiments 2 through 6, Table 5) were examined for TonB$^-$ segregants lacking these markers. In each case, transductants were found that simultaneously lost *trpDCBA-tonB*$^+$ and the transdominant marker. From this we conclude that these transductants are heterogenotes for the loci tested, and therefore that the complete segment *trpD* through *purB* is duplicated. Since *purB* but not *fabD* is cotransducible with *trpDCBA* (Table 5), we also conclude that the duplication is arranged in tandem, although a small region of unduplicated DNA between *purB* and *trpDCBA* cannot be ruled out (see Discussion).

The results of transduction and segregation analysis of several such heterogenotes are presented in Table 6. The position of *galU*$^+$ in strains RM1053-3 and RM1053-4 and the position of *tyrT3* in strains RM621-5 and RM621-7 are clearly indicated by the transduction data. However, the position of *hemA*$^+$ in strains RM1088 and RM1100 is less certain, since the cotransduction frequency of *trpDCBA* and *hemA*$^+$ for strain RM1100 is less than that observed when the *hemA*$^+$/*hemA*$^+$ homogenote MS304 is used as donor (experiment 4, Table 5). The position of *dadR1* in strain RM965 cannot be determined by transduction, but the probable position is indicated in Table 6. The position of *purB*$^+$ indicated for strain RM961 is based on the fact that *purB* is cotransducible with *trpDCBA* but not with the *trp* operon (experiments 6 and 14, Table 5; reference 17).

The results of the segregation experiments indicate that the frequency of loss of a genetic marker in a heterogenote is determined by its position in the duplication (Table 6). That is, markers closely linked to *trpDCBA* are lost more often than those that are not closely linked. For example, in strain RM1053-3, *galU95* is closely linked to *trpDCBA*, but *galU*$^+$ is not; therefore, 91% of the segregants are Gal$^+$ and only 9% are Gal$^-$. Similar results were obtained for all other strains tested except the *hemA* heterogenotes, strains RM1088 and RM1100. In these cases, the data appear biased in favor of *hemA*$^+$ segregants. The fact that the frequency at which a marker is lost is approximately proportional to its genetic distance from *trpDCBA* is consistent with segregation resulting from a single-site crossover that occurs at random in the interval *galU* to *purB*; that is, there are no hot spots of recombination.

**Location of termini of the duplication.** The transduction and segregation data presented in Tables 5 and 6 indicate that one terminus of the duplication is between the normal positions of *purB* and *fabD*. We do not believe *trpDCBA* is fused to *purB*, since a higher frequency (>90%) of cotransduction would be expected. The fact that strains containing the duplication can utilize anthranilic acid to synthe-

TABLE 6. *Transduction and segregation analysis of heterogenotes*

| Strain | Relevant genotype | Transduction[a] | | Segregation[b] | |
|---|---|---|---|---|---|
| | | Marker tested | Percent cotransduction with *trpDCBA* | Marker lost | Percent of total |
| RM1053-3 | Δ(*tonB-trpAE*)*2 galU*$^+$:*trpDCBA galU95* | *galU*$^+$ | 5 (2/37) | *galU95* | 91 (327/360) |
| RM1053-4 | Δ(*tonB-trpAE*)*2 galU95*:*trpDCBA galU*$^+$ | *galU*$^+$ | 79 (22/28) | *galU*$^+$ | 84 (242/288) |
| RM621-5 | Δ(*tonB-trpAE*)*1 tyrT3*:*trpDCBA tyrT*$^+$ | *tyrT3* | 2 (1/64) | *tyrT*$^+$ | 85 (200/235) |
| RM621-7 | Δ(*tonB-trpAE*)*1 tyrT*$^+$:*trpDCBA tyrT3* | *tyrT3* | 50 (8/16) | *tyrT3* | 76 (152/200) |
| RM1088 | Δ(*tonB-trpAE*)*2 hemA*$^+$:*trpDCBA hemA8* | *hemA*$^+$ | 5 (7/144) | *hemA8* | 62 (350/561) |
| RM1100 | Δ(*tonB-trpAE*)*2 hemA8*:*trpDCBA hemA*$^+$ | *hemA*$^+$ | 15 (21/144) | *hemA*$^+$ | 12 (36/288) |
| RM965 | Δ(*tonB-trpAE*)*2 dadR1*:*trpDCBA dadR*$^+$ | NT | NT | *dadR*$^+$ | 24 (70/288) |
| RM961 | Δ(*tonB-trpAE*)*2 purB*$^+$:*trpDCBA purB51* | NT | NT | *purB51* | 12 (67/576) |

[a] Cotransduction was tested using strains in table as donors and appropriate strains as recipients (RM1052 for *galU*$^+$, MS397 for *tyrT3*, RM757 and RM1099 for *hemA*$^+$). All transductants selected on indole-supplemented medium. Δ-Aminolevulinic acid was added to medium when strain RM757 or RM1099 was used as recipient. Transductants with marker tested/total transductants is given in parentheses. NT, Not tested.

[b] TonB$^-$ segregants were selected for resistance to colicin V,B and $\phi$80*vir* on L-broth medium (supplemented with Δ-aminolevulinic acid for strains RM1088 and RM1100 and with adenine for strain RM961). Segregants that lost the indicated marker/total segregants tested is given in parentheses. All segregants were also TrpB$^-$.

size tryptophan indicates that *trpDCBA* specifies the phosphoribosyltransferase activity of the *trpD* polypeptide. This was confirmed by enzyme assays (24). However, since the operator-proximal one-third of *trpD* is not essential for this activity (16), all of *trpD* is not necessarily duplicated. To test this possibility, spot transduction tests were performed using strain MS304 containing *trpDCBA* and mutants altered in *trpE* or *trpD*. The results indicate (Table 7) that the terminus of the duplication occurs at a site that is normally between the most operator-distal mutation (E5947) in *trpE* and the most operator-proximal mutation (D159) in *trpD*.

**Transdominance of *dadR1* over *dadR*$^+$.** Kuhn and Somerville (17) first reported *dadR* mutations that allow certain amino acid auxotrophs to utilize D-amino acids in place of the respective L-amino acids. These workers suggested that *dadR* may be a regulatory gene for D-amino acid deaminases. In the present studies, we found that the mutant locus *dadR1* is transdominant over the wild-type locus *dadR*$^+$. This was determined as follows. The duplicated segment *trpDCBA* was transferred by transduction from strain MS304 into strain RM881 [Δ(*tonB-trpAE*)*2 dadR1*]. Seven of the 150 transductants examined were DadR$^+$ (unable to utilize D-tryptophan), and 143 were DadR$^-$. None of the seven DadR$^+$ transductants gave rise to DadR$^-$ segregants (less than $1/10^8$ cells). TonB$^-$ segregants from 47 of the DadR$^-$ transductants were screened for DadR$^+$ clones. Only 4 of the 47 transductants gave rise to DadR$^+$ segregants. We conclude that these four DadR$^-$ transductants are heterogenotes (*dad1/dadR*$^+$) and that *dadR1* is transdominant to *dadR*$^+$. We also conclude that the seven DadR$^+$ transductants are homogenotes (*dadR*$^+$/*dadR*$^+$) and that the 43 DadR$^-$ transductants that do not yield DadR$^+$ segregants are also homogenotes (*dadR1/dadR1*). The cotransduction frequency (9%) of *trpDCBA* and *dadR*$^+$ (experiment 5, Table 5) is based solely on the 47 DadR$^-$ transductants that were tested for DadR$^+$ segregants. If the frequency (5%) of DadR$^+$ transductants (*dadR*$^+$/*dadR*$^+$ homogenotes) is included, the cotransduction frequency is approximately 14%, which is higher than the frequency (2%) observed for *dadR* and the *trp* operon (experiment 13, Table 5). From this we conclude that *dadR* is closer to *trpDCBA* than to the *trp* operon in its normal position.

TABLE 7. *Mapping of trpDCBA with point mutations in trpE and trpD*[a]

| Point mutation tested | Distance of point mutation from origin of gene[b] | Trp$^+$ transductants obtained with: Strain MS304 (*trpDCBA*) | Strain RM159 (*trpB9578*) |
|---|---|---|---|
| *trpE9777* | 0.55 | − | + |
| *trpE10220* | 6.3 | − | NT |
| *trpE5972* | 6.9 | − | + |
| *trpE22-1* | 7.1 | − | + |
| *trpE5947* | 7.3 | − | + |
| *trpD159* | 0.3 | + | + |
| *trpD233* | 0.44 | + | + |
| *trpD9885* | 1.55 | + | + |

[a] P1*kc*-mediated spot transductions were performed using strain MS304 as donor and as recipient with each *trpE* and *trpD* strain (except D233, which was not used as recipient). The control strain RM159 used as recipient only. NT, Not tested.

[b] Distance from operator-proximal terminus of respective gene given in map units (32).

**Gene conversion between copies of the duplication.** Since one copy of the duplicated region *trpD-purB* is longer than the longest possible bacteriophage P1*kc* transducing fragment, it is very unlikely that both copies of a duplicated genetic marker will be transferred during one transductional event. Therefore, we suggest that the seven DadR$^+$ transductants described above were derived through an intermediate DadR$^-$ heterogenote (*dadR1/dadR*$^+$) that converted to the DadR$^+$ homogenote (*dadR*$^+$/*dadR*$^+$). A similar phenomenon was observed when *trpDCBA* was transferred by transduction (donor strain MS304) to a *tonB-trpAE* deletion strain RM594 containing *tryT3*. As expected, most of the transductants were TyrT$^-$, since *tyrT3* is transdominant. However, a small fraction (4 out of 94) were TyrT$^+$ and therefore must be homogenotes (*tyrT*$^+$/*tyrT*$^+$). This finding suggests that these transductants were also derived through a heterogenote (*tyrT*$^+$/*tyrT3*). One other example of conversion was observed when a Hem$^+$ heterogenote (*hemA8/hemA*$^+$) gave rise spontaneously to Hem$^-$ homogenotes (*hemA8/hemA8*) at a low frequency (0.3% of colonies tested). These results indicate that unequal genetic exchange occurs between the copies of the duplicated material.

## DISCUSSION

Genetic and segregation analysis of *E. coli* strains containing a partial duplication of the *trp* operon allows us to conclude that the duplication includes *trpD* through *purB* and that it is arranged in tandem in the chromosome. A diagram of the chromosome structure in this region for strains RM213 and MS304 is given in Fig. 1. Actually, our data do not exclude the possibility that a region of unduplicated material occurs between *purB* and *trpDCBA*, since the cotransduction frequency of these markers is

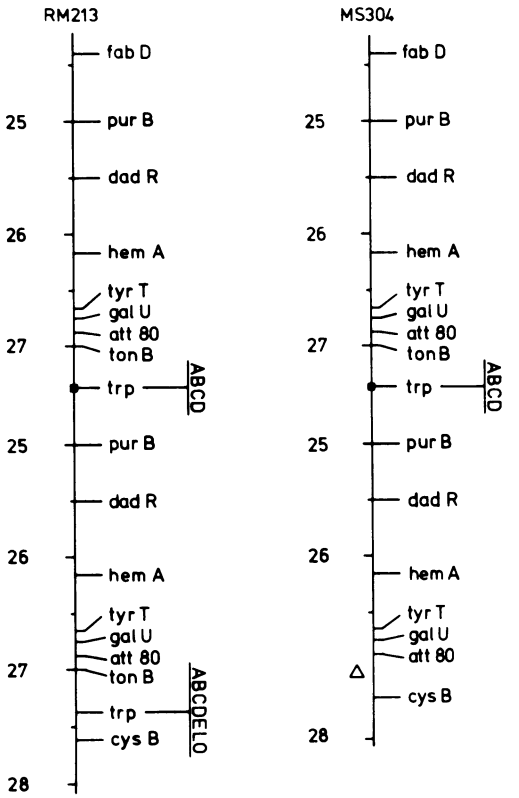


FIG. 1. *Genetic map of chromosomal region containing duplication in strains RM213 and MS304. The genetic structure of the cysB-fabD region in strains RM213 (left) and MS304 (right) is depicted by vertical lines. Strain RM213 contains a tandem duplication of the trpD-purB region (repeat point indicated by ■). The duplicated region in strain MS304 was derived by transduction from strain RM213 (experiment 1, Table 2). As a result of the tonB-trpAE deletion (indicated by Δ) in strain MS304, two copies of the att80-purB region are separated by a single copy of the trpD-tonB region. The positions of genetic loci are based on the E. coli K-12 linkage map (3), which is calibrated in minutes (numbers in figure), and on data presented in this paper. The trpL region has been described (15).*

about 36%. There are no known genetic loci between *purB* and *trpDCBA*, and thus this question could be answered unequivocally only by physical or chemical means. To our knowledge, this possibility has not been excluded for other duplications reported to be tandem. In strain MS304 containing the deletion Δ(*tonB-trpAE*)*1* in one segment of the duplication, the chromosome structure is that of a translocation duplication, since the region *trpD-tonB* actually occurs in single copy between duplicate copies of the *att80-purB* region.

In several cases we have noticed that the segregation frequency of *trpDCBA* (determined as described previously [24]) in *tonB-trp* deletion strains (e.g., MS304) is consistently three- to fivefold lower than in the parental strain RM213 (Simonian and Mosteller, unpublished observations). The cause of this effect has not been determined. However, it may be due to the altered structure of the duplicated region in these strains (Fig. 1) or, alternatively, to differences in relative growth rates of the parental and segregant strains.

Hill et al. (12) suggested that tandem duplications which are longer than the maximum size of a transducing fragment can be generated in a recipient during transduction if the repeat point of the duplication is transferred and a three-point recombination event occurs between the transferred fragment and two copies of the recipient chromosome. Our data are consistent with this suggestion and, in addition, indicate that this process occurs at a frequency equal to that observed for normal transduction (see Table 2). This implies that the rate-limiting step in each case may be the same. For example, both may require the replicating fork of the chromosome where two copies of a given region must be in close proximity for at least a brief period of time. Since *trpDCBA*-containing transductants are not generated when the parental strain RM106 is used as donor (experiment 3, Table 2), we conclude that *trpDCBA*-containing transductants obtained when strain RM213 or strain MS304 is used as donor do not result from preexisting duplications in the recipient as described for the *his* operon in *S. typhimurium* (1).

The fact that cotransduction frequencies between *trpDCBA* and other genetic loci (*tonB*, *galU*, *tyrT*, and *hemA*) are very similar to those observed for the *trp* operon and the same markers (Table 5) indicates that the mechanism of integrating a duplication during transduction does not alter normal genetic linkage. These findings also suggest that the duplicated region *trpD-hemA* does not contain a deletion of significant size that would increase the linkage observed. Our data do not exclude the possibility of small deletions in the duplicated *hemA-purB* region, although the *dadR* locus in this region is known to be duplicated since heterogenotes containing *dadR1* and *dadR*$^+$ were found.

The initial step in segregation of duplications is believed to be a single-site recombination event between homologous duplicated segments followed by loss or dilution of DNA containing one copy of the region that was duplicated (11, 12). Although this model has not been proven by direct physical means, it seems the most plausible explanation. The segregation analysis

of heterogenotes presented in Table 6 is consistent with this model and, in addition, shows that the site of recombination occurs at random in the interval *galU-purB*. This is demonstrated by the fact that the frequency at which a specific marker is lost during segregation is approximately proportional to its distance from the terminus of the duplicated region (Fig. 1). Because *tonB-trp* deletion strains were used, this conclusion does not necessarily apply to the frequency of recombination at sites in the *tonB-trp* region in nondeletion strains.

The published map of the *E. coli* K-12 chromosome shows *dadR* about 0.2 min from *hemA* and 1.0 min from *purB* (3). Our transduction data indicate that *dadR* is closer to *purB* than to *hemA* (experiments 15 and 17, Table 5). The cotransduction frequency of *trpDCBA* with *dadR* (see Results and experiment 5, Table 5) is also consistent with *dadR* being closer to *purB* than indicated by the map (3).

The fact that *dadR1/dadR*$^+$ heterogenotes are phenotypically DadR$^-$ (able to utilize D-amino acids [17]) demonstrates that *dadR1* is transdominant to *dadR*$^+$. This finding indicates that the phenotype of *dadR1* mutants results from acquisition of a *dadR1*-specified function and not from loss of a *dadR*$^+$-specified product.

In several cases mentioned in Results, we observed events that can best be explained by conversion of heterogenotes to homogenotes. This process is probably analogous to a similar phenomenon, called homogenotization, that occurs between F′ plasmids and the host chromosome (20). In both cases, a nonreciprocal recombination event apparently occurs between two copies of homologous DNA. When studying segregation of heterogenotes, one should be careful not to examine only one genetic marker since apparent segregants could arise by either segregation or homogenotization. It should be mentioned that this is not a problem when segregants are selected as described in Table 6, since the *tonB-trpAE* deletions in these strains extend beyond one terminus of the duplicated region (Fig. 1). In this situation, there is duplicated DNA on only one side of the deletion, and therefore homologous recombination cannot result in homogenotes containing two copies of the deletion.

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## LITERATURE CITED

1. **Ames, B. N., P. E. Hartman, and F. Jacob.** 1963. Chromosomal alterations affecting the regulation of histidine biosynthetic enzymes in *Salmonella*. J. Mol. Biol. **7**:23–42.
2. **Anderson, R. P., C. G. Miller, and J. R. Roth.** 1976. Tandem duplications of the histidine operon observed following generalized transduction in *Salmonella typhimurium*. J. Mol. Biol. **105**:201–218.
3. **Bachmann, B. J., K. B. Low, and A. L. Taylor.** 1976. Recalibrated linkage map of *Escherichia coli* K-12. Bacteriol. Rev. **40**:116–167.
4. **Beeftinck, F., R. Cunin, and N. Glansdorff.** 1974. Arginine gene duplications in recombination proficient and deficient strains of *Escherichia coli* K-12. Mol. Gen. Genet. **132**:241–253.
5. **Coukell, M. B., and C. Yanofsky.** 1970. Increased frequency of deletions in DNA polymerase mutants of *Escherichia coli*. Nature (London) **228**:633–635.
6. **Cunin, R., D. Elseviers, and N. Glansdorff.** 1970. *De novo* duplication versus reactivation of cryptic genes in *Escherichia coli* K-12. Mol. Gen. Genet. **108**:154–157.
7. **Folk, W. R., and P. Berg.** 1971. Duplication of the structural gene for glycyl-transfer RNA synthesis in *Escherichia coli*. J. Mol. Biol. **58**:595–610.
8. **Glansdorff, N., and G. Sand.** 1968. Duplication of a gene belonging to an arginine operon of *Escherichia coli* K-12. Genetics **60**:257–268.
9. **Hershey, A. D.** 1955. An upper limit to the protein content of the germinal substance of bacteriophage T2. Virology **1**:108–127.
10. **Hill, C. W., and P. Combriato.** 1973. Genetic duplications induced at very high frequency by ultraviolet irradiation in *Escherichia coli*. Mol. Gen. Genet. **127**:197–214.
11. **Hill, C. W., F. Foulds, L. Soll, and P. Berg.** 1969. Instability of a missense suppressor resulting from a duplication of genetic material. J. Mol. Biol. **39**:563–581.
12. **Hill, C. W., D. Schiffer, and P. Berg.** 1969. Transduction of merodiploidy: induced duplication of recipient genes. J. Bacteriol. **99**:274–278.
13. **Horiuchi, T., S. Horiuchi, and A. Novick.** 1963. The genetic basis of hyper-synthesis of β-galactosidase. Genetics **48**:157–169.
14. **Jackson, E. N., and C. Yanofsky.** 1973. Duplication-translocations of tryptophan operon genes in *Escherichia coli*. J. Bacteriol. **116**:33–40.
15. **Jackson, E. N., and C. Yanofsky.** 1973. The region between the operator and first structural gene of the tryptophan operon of *Escherichia coli* may have a regulatory function. J. Mol. Biol. **76**:89–101.
16. **Jackson, E. N., and C. Yanofsky.** 1974. Localization of two functions of the phosphoribosyl anthranilate transferase of *Escherichia coli* to distinct regions of the polypeptide chain. J. Bacteriol. **117**:502–508.
17. **Kuhn, J., and R. L. Somerville.** 1971. Mutant strains of *Escherichia coli* K-12 that use D-amino acids. Proc. Natl. Acad. Sci. U.S.A. **68**:2484–2487.
18. **Lennox, E. S.** 1955. Transduction of linked genetic characters of the host by bacteriophage P1. Virology **1**:190–206.
19. **Levinthal, M., and Y. Len.** 1972. The pi-histidine factor of *Salmonella typhimurium*: a demonstration that pi-histidine factor integrates into the chromosome. J. Bacteriol. **109**:993–1000.
20. **Low, K. B.** 1972. *Escherichia coli* K-12 F-prime factors, old and new. Bacteriol. Rev. **36**:587–607.
21. **Miller, C. G., and J. R. Roth.** 1971. Recessive-lethal nonsense suppressors in *Salmonella typhimurium*. J. Mol. Biol. **59**:63–75.
22. **Russell, R. L., J. N. Abelson, A. Landy, M. L. Gefter, S. Brenner, and J. D. Smith.** 1970. Duplicate genes

for tyrosine transfer RNA in *Escherichia coli*. J. Mol. Biol. **47**:1-13.

23. **Semple, K. S., and D. F. Silbert.** 1975. Mapping of the *fabD* locus for fatty acid biosynthesis in *Escherichia coli*. J. Bacteriol. **121**:1036-1046.
24. **Simonian, M. H., and R. D. Mosteller.** 1976. Increased loss of duplicated genes in streptomycin-resistant (*strA*) mutants of *Escherichia coli* K-12. J. Bacteriol. **125**:382-384.
25. **Stodolsky, M.** 1974. Recipient gene duplication during generalized transduction. Genetics **78**:809-822.
26. **Straus, D. S.** 1974. Induction by mutagens of tandem gene duplications in the *glyS* region of the *Escherichia coli* chromosome. Genetics **78**:823-830.
27. **Straus, D. S., and G. R. Hoffman.** 1975. Selection for a large genetic duplication in *Salmonella typhimurium*. Genetics **80**:227-237.
28. **Straus, D. S., and L. D. Straus.** 1976. Large overlapping tandem genetic duplications in *Salmonella typhimurium*. J. Mol. Biol. **103**:143-153.
29. **Vogel, H., and D. M. Bonner.** 1956. Acetylornithinase of *Escherichia coli*: partial purification and some properties. J. Biol. Chem. **218**:97-106.
30. **Wang, C. C., and A. Newton.** 1969. Iron transport in *Escherichia coli*: relationship between chromium sensitivity and high iron requirement in mutants of *Escherichia coli*. J. Bacteriol. **98**:1135-1141.
31. **Waring, W. S., and C. H. Werkman.** 1942. Growth of bacteria in an iron-free medium. Arch. Biochem. **1**:303-310.
32. **Yanofsky, C., V. Horn, M. Bonner, and S. Stasiowski.** 1971. Polarity and enzyme functions in mutants of the first three genes of the tryptophan operon of *Escherichia coli*. Genetics **69**:409-433.