SELECTION FOR A LARGE GENETIC DUPLICATION IN *SALMONELLA TYPHIMURIUM*[1]

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ABSTRACT

Salmonella typhimurium strains containing a duplication of nearly a third of the genome have been isolated by a simple procedure involving selection for improved utilization of L-malate as sole carbon source. The duplication occurs at a very high spontaneous frequency. Strains containing the duplication can be isolated selectively on malate medium, or by a non-selective procedure involving Hfr conjugation. When strains containing the duplication are maintained on non-selective medium, the duplication is readily lost. Genetic evidence suggests that the duplication is chromosomal and tandem. The fact that the *recA* gene is included in the duplication has been used to obtain evidence that the *recA1* marker is recessive to its wild-type allele. Unlike tandem duplications previously described in *E. coli*, the duplication described in this report appears to have unique endpoints.

TANDEM gene duplications have been found to arise at high spontaneous frequencies in bacteria (FOLK and BERG 1971; GLANSDORFF and SAND 1968; HILL *et al.* 1969; HILL and COMBRIATO 1973). In *Escherichia coli*, tandem duplications covering as much as six percent of the genome have been described (FOLK and BERG 1971; HILL and COMBRIATO 1973). It has been proposed previously that such duplications are formed by aberrant recombinational events in which non-homologous DNA sequences are joined (reviewed by FRANKLIN 1971). In this paper we describe and genetically characterize strains of *Salmonella typhimurium* which contain a duplication of nearly one-third of the chromosome. Based upon genetic experiments, we propose that the duplication is tandem. The length of the duplication is the same in all strains examined, suggesting that there might be regions of genetic homology at the endpoints of the duplication.

MATERIALS AND METHODS

Bacterial strains: All strains are derived from the wild-type strains LT-2 and LT-7 of *S. typhimurium*. Many of the singly marked auxotrophic strains were kindly provided to us by DRS.

[1] Abbreviations: Mte$^+$: fast growth on L-malate as a carbon source.
Mte$^-$: slow growth on L-malate as a carbon source.

P. E. HARTMAN and B. N. AMES. The genotypes of all multiply marked strains are given in the text.

Media and genetic methods: Minimal glucose medium consisted of medium E (VOGEL and BONNER 1956) plus 2% glucose. Minimal malate medium contained minimal salts medium (GUTNICK *et al.* 1969), supplemented with 0.2% L-malate as sole carbon source and 10 mM NH_4Cl. All transductions were performed with P22-L3 phage (SMITH and LEVINE 1967). Conjugations were performed by plating 10^3 to 10^6 stationary phase donor cells with 10^8 recipient cells on solid selective medium.

Selection for malate fast-growers: To select for malate fast-growers, 10^{-4} ml and 10^{-3} ml aliquots of stationary phase nutrient broth cultures were spread on solid minimal malate medium in 15 × 100 mm petri dishes. Fast-growing colonies were observed through a dissecting microscope after incubation times ranging from 24 to 72 hours, depending upon the bacterial strain. Most fast-growers have a rough colony morphology an are easily detected against a relatively light layer of background growth. When more than 10^6 cells (about 10^{-3} ml) are spread per plate, fast-growers are often obscured by background growth. Occasional nutrient broth cultures contain unusually high numbers of fast-growers. When such cultures are plated on solid selective media, it is necessary to plate at low densities (10^5 or fewer cells per dish) to avoid confluent growth. In some strains (Table 1) heavy background growth completely prevents isolation of fast-growers even at low cell concentrations.

RESULTS

Selection for fast growth on L-malate

Salmonella typhimurium strain LT-2 grows extremely slowly on L-malate as a sole carbon source. Unexpectedly, it grows faster on acetate even though incorporation of acetate requires metabolism to L-malate *via* the glyoxylate cycle (MANDELSTAM and MCQUILLEN 1968). It seems likely, therefore, that the poor growth of Salmonella on L-malate relative to that on acetate is due to inefficient transport of L-malate into the cells.

When strain LT-2 is plated on solid minimal medium containing L-malate as the sole carbon source, large fast-growing colonies (Mte$^+$) appear above a leaky background of very small colonies after 24 to 72 hours at 37°.[1] Fast-growing colonies are detected at the high frequency of 4×10^{-5} to 4×10^{-4} per viable cell plated.

Colonies which grow well on malate (Mte$^+$) may be classified into at least two categories. The great majority of Mte$^+$ colonies are of rough colony morphology on both minimal malate and minimal glucose medium. These colonies are genetically unstable and readily yield segregants which grow poorly on malate (Mte$^-$). The Mte$^-$ segregants are easily recognized as small smooth colonies on minimal malate medium (Figure 1). Cultures of unstable Mte$^+$ strains grown in liquid minimal malate medium generally contain about 5% to 10% Mte$^-$ segregants. The number of Mte$^-$ segregants stays fairly constant when Mte$^+$ cells are subcultured into malate medium, although some cultures with as many as 30% Mte$^-$ segregants are observed. When cells are transferred from liquid malate medium to non-selective medium (nutrient broth), the frequency of Mte$^+$ cells decreases by two to five percent per generation.

[1] First pointed out to us by L. KIER.

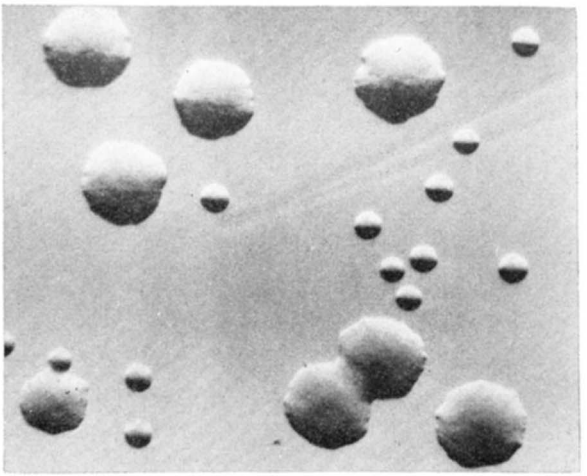

FIGURE 1.—Large rough colonies of unstable malate fast-growers derived from wild-type strain LT-2, and small smooth segregant colonies. The colonies are growing on minimal malate medium. The larger colonies are approximately 1 mm in diameter.

In addition to the unstable rough colonies, a much smaller class of genetically stable Mte$^+$ colonies is also selected on minimal malate medium. These colonies, which we have not further characterized, are easily distinguished from the unstable Mte$^+$ colonies because they have a smooth colony morphology.

There is considerable variation among strains of *Salmonella typhimurium* with respect to selection for fast growth on L-malate. About one-third of the strains that we tested (Table 1) grew so well on minimal malate medium that it was not possible to isolate Mte$^+$ colonies. In the majority of strains, however, Mte$^+$ colonies could be detected against various levels of background growth. Two strains in which Mte$^+$ colonies can be identified with particular ease are *hisG46* and SB109 (*ilvA401 metE338 strA ara9*). The variation among strains is difficult to understand since all of the strains tested are derived from wild-type strains LT-2 or LT-7, both of which grow poorly on malate.

Differences in ability to grow on malate cannot be attributed to known genetic markers in our strains. Strain *cysG439*, for example, grows too well on malate to isolate Mte$^+$ colonies in this strain. When the *cysG439* marker is transduced into the Mte$^-$ strain TS67 (*argG459*), however, recombinants containing the *cysG439* allele grow poorly on L-malate and readily yield Mte$^+$ colonies. There is apparently great heterogeneity among commonly used laboratory strains of Salmonella at some locus or loci controlling the ability to grow on L-malate.

Evidence for a large genetic duplication in Mte$^+$ strains

The high spontaneous frequency of occurrence and instability of the Mte$^+$ strains suggested that these strains might contain a tandem duplication of genetic material. Tandem duplications occur at spontaneous frequencies much higher than point mutations in *E. coli* (FOLK and BERG 1971; GLANSDORFF and SAND

TABLE 1

Comparison of strains for selection of malate fast-growers

Strains in which Mte+ colonies can be selected	
LT2	*pheA33*
	TS70 (*cysA2601*)
pyrE125	*glpT112*
TS311 (*pyrE125 cysA2602*)	*glpT113*
TS2 (*hisΔ63 xyl301*)	*hisG46*
TR970 (*hisO1242 hisC3737*)	*trp-962*
glpD107	*nicA502*
TS81 (*cysG439*)	*thrB13*
TS67 (*argG459*)	*thrA11*
argE116	*metA6*
metC52	SB109 (*metE338 ilvA401 strA ara9*)
lys-507	SA962 (Hfr K16 *thrA49 leuBCD39 ara9*)
TS129 (*cysG439 thy-1631*)	SU354 (HfrB2 metA22 [P22+])
TS331 (*hisG46 cysA2602 xyl306*)	
Strains in which Mte+ colonies cannot be selected	
lys-505	*thrB23*
trpD9	*phe-3*
cysG439	*phe-34*
metC30	*phe-502*
argE36	*purC2*
trpA278	*purI305*
trpC109	*purF145*
argA85	SA654 (Hfr K9 *thr-9*)
thrA9	

1968; HILL *et al.* 1969; HILL and COMBRIATO 1973) and are unstable because they can be lost by a single recombinational event.

Since utilization of L-malate in Salmonella appeared to be limited by transport it seemed plausible that the unstable Mte+ strains contained a tandem duplication which included the dicarboxylic acid permease gene, *dct* (KAY and KORNBERG 1971; LO, RAYMAN and SANWAL 1972). The increased gene dosage for *dct* provided by such a duplication might increase malate transport enough to permit faster growth on minimal malate medium.

Beginning with genes which map near *dct*, a simple genetic test was employed to detect merodiploidy in Mte+ strains. This technique, which has also been used for mapping the extent of *glyS* and *glyT* tandem duplications in *E. coli* (FOLK and BERG 1971; HILL and COMBRIATO 1973), is based upon the detection of heterozygosity for the gene being tested. Heterozygotes are constructed by selecting a duplication in a strain carrying a recessive marker and then restoring the wild-type phenotype by transduction, conjugation, or reversion. If the reversion method is used, it is essential that all revertants be true back mutants or intragenic suppressors because intergenic suppressors may be distant from the gene being tested. Recovery of the recessive allele in Mte- segregants

indicates that the Mte$^+$ strain is in fact merodiploid for the marker in question. If the gene is not duplicated, recovery of the recessive allele from a heterozygote does not occur.

A typical experiment for the detection of merodiploidy was that conducted with strain TS311 (*cysA pyrE125*). A number of independent Mte$^+$ derivatives of strain TS311 were isolated on minimal malate medium supplemented with uracil and cysteine. The Mte$^+$ strains were made Pyr$^+$ by transduction on minimal malate medium supplemented with cysteine. Similarly, Cys$^+$ transductants and revertants were isolated on medium supplemented with uracil. The recombinants or revertants were restreaked twice on malate medium, after which a single Mte$^+$ colony from each strain was grown in nutrient broth. Cells from the broth culture were plated on minimal malate medium supplemented with cysteine and uracil. About fifty smooth Mte$^-$ segregants were then tested for cysteine or uracil requirements. In all strains, both *cysA*$^-$ and *cysA*$^+$ colonies were found among the Mte$^-$ segregants derived from the Mte$^+$ Cys$^+$ transductants and revertants. Similarly, both *pyrE*$^-$ and *pyrE*$^+$ segregants were obtained from the Pyr$^+$ transductants in the duplication strain. Recovery of the *cysA*$^-$ or *pyrE*$^-$ alleles from the Cys$^+$ or Pyr$^+$ transductants and revertants in the Mte$^+$ strain indicated that those transductants and revertants were actually *cysA*$^+$/*cysA*$^-$ or *pyrE*$^+$/*pyrE*$^-$ merodiploids.

Tests similar to that described for strain TS311 were conducted for 74 independently isolated Mte$^+$ strains. In these experiments, 24 different genes mapping around the chromosome were examined for merodiploidy. The results (Table 2) indicate that the Mte$^+$ strains contain a duplication which is not restricted to the region surrounding *dct*, but rather includes nearly a third of the Salmonella genome. As shown in Figure 2, the duplication has been found to extend from *glpT* through *pyrE*, a region of approximately 42 minutes of the chromosome.

The endpoints of the duplicated region in Mte$^+$ strains are distinctly nonrandom. In testing at least seven independently isolated Mte$^+$ derivatives from each strain it was found that the widely separated markers *pyrE* and *glpT* were in all cases duplicated, whereas *ilvA* and *hisG* were never duplicated. One end of the duplication, therefore, lies in the six-minute interval between *pyrE* and *ilvA*. The other end point is between *glpT* and *hisG*, an interval of approximately nine minutes.

Evidence that the duplication is chromosomal and tandem

The pattern of segregation of two alleles from heterozygous merodiploids strongly suggests that the duplication in the Mte$^+$ strains is chromosomal rather than episomal. Following loss of the duplication, the two alleles are commonly recovered at approximately equal frequencies, and their ratio very rarely exceeds 20:1. If the duplication were episomal, recovery of both alleles at high frequency would be very unlikely. Linkage of the duplication with the chromosome is also suggested by our observation (see below) that the duplication is transferred in Hfr conjugations.

TABLE 2

Tests for merodiploidy

Strain	Locus tested for duplication	Method for heterozygote construction*	Number of independent Mte+ isolates in which gene is duplicated
pyrE125	*pyrE*†	T	5/5
TS311 (*pyrE125, cysA2603*)	*pyrE*†	T	10/10
TS2 (*xyl301, hisΔ63*)	*xyl*‡	T	1/1
TS2 (*xyl301, hisΔ63*)	*glyS*§	T	1/1
TR970 (*hisO1242 hisC3737*)	*sufA*¶	T	4/4
glpD107	*glpD*‡	R	1/1
TS81 (*cysG439*)	*cysG*†	T	3/3
TS67 (*argG459*)	*argG*‖	T	2/2
TS67 (*argG459*)	*strA*‖	T	2/2
argE116	*argE*†	T	1/1
metC52	*metC*†	T	1/1
lys-507	*lys*†	T	1/1
TS129 (*cysG439 thy-1631*)	*cysG***	T	1/1
TS129 (*cysG439 thy-1631*)	*thy***	C	1/1
TS129 (*cysG439 thy-1631*)	*recA***	C	1/1
pheA33	*pheA*†	T	4/4
TS70 (*cysA2601*)	*cysA*†	T	3/3
TS311 (*pyrE125 cysA2603*)	*cysA*†	T,R	10/10
glpT112	*glpT*‡	T	3/3
glpT113	*glpT*‡	T	4/4
hisG46	*hisG*†	T	0/9
trp-962	*trp*†	T	0/1
nicA502	*nicA*†	T	0/1
thrB13	*thrB*†	R	0/1
thrA11	*thrA*†	T,R	0/1
metA6	*metA*†	T	0/4
SB109 (*metE338 ilvA401 strA ara9*)	*metE*†	T,C	0/15
SB109 (*metE338 ilvA401 strA ara9*)	*ilvA*†	T,C	0/13

* Three methods were used for the construction of heterozygotes in duplication strains: transduction (T), conjugation (C), and reversion (R).

† Unstable Mte+ derivatives of auxotrophic strains were made prototropic on minimal malate medium by the method indicated, streaked twice on minimal malate medium and grown up in nutrient broth. Mte− segregants were tested for auxotrophy by replicating on supplemented and unsupplemented minimal glucose medium.

‡ Same as above, except that heterozygotes were constructed on solid medium containing D-xylose (0.3%) or D,L-α-glycerol phosphate (0.4%) as the sole carbon source.

§ Phage grown on the glycine-requiring glycyl-tRNA synthetase mutant TS1 (*hisΔ63 glyS141*) were used to transduce the Mte+ strain TS146 (*hisΔ63 xyl301/xyl301*) to growth on minimal xylose medium, and Mte+ Xyl+ transductants were scored for glycine requirement. Even though *glyS* is 75% cotransducible with *xyl* (D. STRAUS, manuscript in preparation), no Gly− transductants were obtained in this cross (0/46). However, most recombinants (38/46) gave rise to both Gly− Mte− and Gly+ Mte− segregants. This experiment indicates duplication of *glyS* in TS146.

¶ Phage grown on strain TR1458 (*hisO1242 hisC3737 sufA7*) were used to transduce Mte+ derivatives of strain TR970 to His+ on minimal malate medium. Transductants became His+ through receipt of the *sufA* allele. The Mte− segregants obtained from His+ Mte+ transductants were of both His+ and His− types, indicating duplication of *sufA*.

‖ Unstable Mte+ derivatives of strain TS67 (*argG459*) were transduced to *argG*+ on minimal glucose medium with phage grown on strain SB109, which contains a *strA* mutation. Recombinants receiving the *strA* mutation readily yielded Arg+ Str^r and Arg− Str^s Mte− segregants, as well as at least one Arg+ Str^s recombinant type segregant.

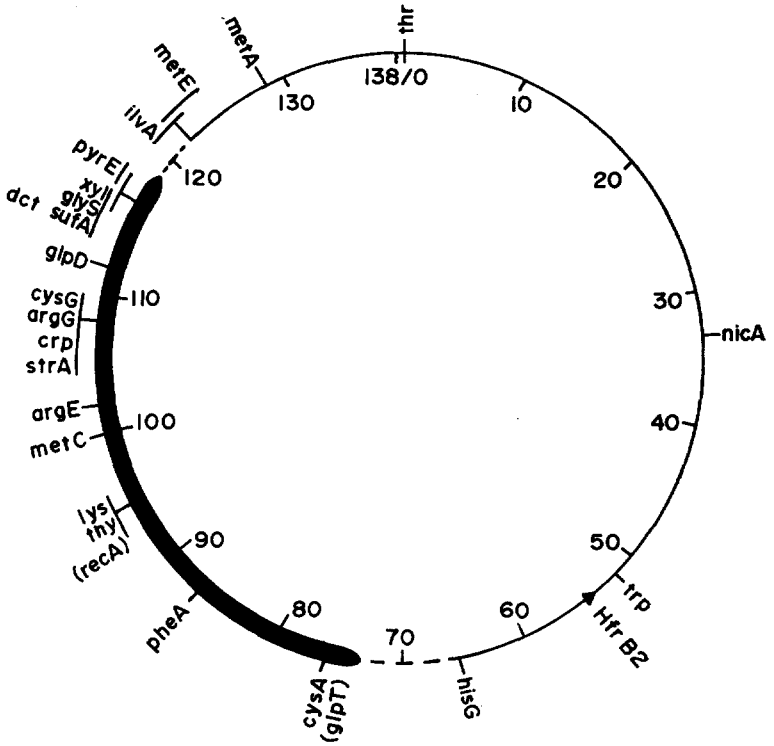

FIGURE 2.—Map of the *Salmonella typhimurium* chromosome (SANDERSON 1972) showing the extent of the duplication in Mte+ strains. The duplicated region is shown by a heavy line, and the unmapped region by a broken line.

Among possible chromosomal duplications, the high frequency of duplication loss observed in Mte+ strains is most consistent with a tandem association. In tandem gene duplications, a single recombinational event can cause loss of the duplication; other types of duplications (e.g. translocated, inverted, etc.) are not as readily lost. A strong basis for concluding that the duplication is tandem would be provided by a demonstration that duplication-loss is dependent upon *recA*-mediated general recombination (FOLK and BERG 1971). Inclusion of *recA* in the *Mte*+ duplication, however, makes it difficult to construct recombination-less derivatives of the duplication strains.

Dominance of recA

Merodiploidy for a large region of the chromosome can be quite useful in genetic studies. We have taken advantage of the Mte+ duplication to determine

** Strain TS150, an unstable Mte+ derivative of TS129, was made heterozygous for *thy* and *recA* by conjugation with strain DB43 (HfrB2 *metA22 strA recA1*), and for *cysG* by transduction. Heterozygosity for *recA* was indicated by the occurrence of both RecA+ Mte- and RecA- Mte- segregants. All eight possible combinations of the three markers were obtained among Mte- segregants, indicating the *thy* and *cysG* were also duplicated.

the dominance of the *recA* marker. Previous studies of the dominance of *recA* in *E. coli* have been inconclusive because of the difficulty in obtaining F′ factors carrying *recA* (CLARK 1971). That *recA* is recessive was demonstrated by an experiment in which *thy*$^+$ exconjugants were obtained from a cross of donor strain DB43 (HfrB2 *metA22 recA1 strA*) and the Mte$^+$ duplication strain TS150 (*cysG439 thy-1631 recA*$^+$/*cysG439 thy-1631 recA*$^+$) on minimal malate medium supplemented with cysteine. All of the Cys$^-$ Thy$^+$ Mte$^+$ exconjugants gave rise to Mte$^-$ segregants of both the *thy*$^+$ and *thy*$^-$ types, demonstrating duplication of *thy*. Many of the Mte$^+$ exconjugants were also heterozygous for *recA*, as evidenced by their segregation of *recA*$^+$ and *recA*$^-$ colonies. Recombination proficiency of these *cysG439 thy*$^+$ *recA1*/*cysG439 thy-1631 recA*$^+$ heterozygotes was indicated by their ability to act as recipients in transductions in which *cysA*$^+$*cysA439* recombinants were selected on minimal malate medium. The lack of UV sensitivity of the heterozygotes also supports the conclusion that *recA*$^+$ is dominant to *recA1* in Mte$^+$ merodiploids.

Non-selective method of isolating Mte$^+$ duplications

In addition to selecting Mte$^+$ duplications by plating on minimal malate medium, it is possible to obtain Mte$^+$ duplications by a totally non-selective method. This method is based upon the characteristic rough colony morphology of Mte$^+$ merodiploids. We observed that approximately 0.7% of the His$^+$ exconjugants from a cross of SU354 (HrfB2 *metA22 strA*, orientation shown in Figure 1) and *hisG46* on minimal glucose medium had a rough morphology. When these rough colonies were streaked on minimal malate medium, about half were identical in growth characteristics and morphology to unstable Mte$^+$ merodiploids selected for growth on malate.

To demonstrate that the rough recombinants obtained on glucose medium are in fact Mte$^+$ merodiploids, we crossed strain SU354 into strain TS331 (*xyl306 cysA2602 hisG46*). Strain TS331 was chosen as the recipient for this cross because two of its genetic markers (*xyl*, *cysA*) would be included in a Mte$^+$ duplication, whereas the third (*hisG*) would not. Approximately 0.4% of the His$^+$ recombinants selected on minimal glucose medium supplemented with cysteine were of the rough morphology. When 21 of the rough His$^+$ recombinants were tested on minimal malate medium supplemented with cysteine, 14 proved to be unstably Mte$^+$. These Mte$^+$ exconjugants could be divided into three classes: three of the exconjugants were Xyl$^+$ Cys$^+$ His$^+$, nine were Xyl$^-$Cys$^+$ His$^+$, and two were Xyl$^-$ Cys$^-$ His$^+$. By segregation analysis, it was possible to show that the three classes were genotypically *xyl*$^-$ *cys*$^-$/*xyl*$^+$ *cys*$^+$ *his*$^+$, *xyl*$^-$ *cys*$^-$/*xyl*$^-$ *cys*$^+$ *his*$^+$, and *xyl*$^-$ *cys*$^-$/*xyl*$^-$ *cys*$^-$ *his*$^+$.[2] These results indicated merodiploidy of *cysA* and *xyl*, but not of *his*. The Mte$^+$ duplications obtained after conjugation on glucose therefore appear to cover the same region as those selected on minimal malate medium.

[2] Two of the Xyl$^-$ Cys$^+$ His$^+$ types may be *xyl*$^-$ *cys*$^+$/*xyl*$^-$ *cys*$^+$ *his*$^+$. There is no way of ascertaining whether *cysA* is actually merodiploid in these two strains.

Transmission of Mte+ duplications in conjugation

To determine whether the duplication can be transferred in conjugation, we selected an unstable malate fast-grower in an Hfr strain, SU354. The resultant strain (TS333) was then crossed into strain *hisG46*, selecting for His+ recombinants on minimal malate medium. As noted above, a few unstable Mte+ His+ recombinants are observed even when neither the donor nor the recipient is Mte+. However, the number of unstable Mte+ His+ recombinants obtained in the cross using strain TS333 as donor was at least ten times greater than that observed in the same cross using strain SU354. This experiment indicates that the duplication is transferred in conjugation.

DISCUSSION

The slower growth of Salmonella on L-malate than on acetate suggests that transport is limiting in the utilization of L-malate as a carbon source. The chromosomal duplication conferring malate fast-growth includes *dct*, which is the structural gene for the dicarboxylic acid permease (KAY and KORNBERG 1971; LO, RAYMAN and SANWAL 1972). In addition, it also includes the gene for the cyclic AMP binding protein (*crp*), which exerts positive control over induction of the dicarboxylic acid permease (LO, RAYMAN and SANWAL 1972). It seems likely that the increased gene dosage of either or both of these two loci could lead to increased synthesis of dicarboxylic acid permease and thereby to improved transport of L-malate. It should be noted that although the properties of the malate fast-growers are most simply explained by tandem duplication, we cannot rule out the possibility of tandem triplication or higher amplification.

The Mte+ duplication extends over a much larger region of the chromosome than would be necessary for duplicating only *dct* and *crp*. It is possible that duplication of genes other than *dct* and *crp* is required to confer a selective advantage on malate medium. Alternatively, the non-randomness of the endpoints of the duplication raises the interesting possibility that the duplication is formed by unequal crossing over between related DNA sequences, comparable to the way in which duplications are formed in higher organisms (reviewed by OHNO 1970). For example, if there is a chromosomal segment between *pyrE* and *ilvA* that is complementary to a segment between *glpT* and *hisG*, crossing over might occur frequently between these two regions, resulting in the formation of a tandem duplication. In this case, the great length of the duplication would simply be a consequence of the mechanism by which it is formed. To our knowledge, this is the first instance in which this type of crossover has been described in bacteria. One other example of unequal crossing over has been observed in *E. coli*, but this involved crossing over within a much shorter tandem duplication to form single-gene and three-gene derivatives (RUSSELL *et al.* 1970).

Three lines of evidence suggest that the duplication is chromosomal and tandem: it is highly unstable, can be transferred in conjugation, and segregates both copies of duplicated genes at high frequency when the duplication is lost. The Mte+ duplication is much longer than any other tandem duplications pre-

viously described in bacteria. Apparently, a chromosome considerably longer than normal can be replicated without having lethal effects on the normal synchrony of cellular division.

The high frequency of occurrence, and genetic instability of tandem duplications make them an interesting class of mutations from an evolutionary viewpoint. One can imagine many natural growth conditions in which it would be selectively advantageous for bacteria to be able to amplify a portion of their genome. Compared to other types of mutations affecting gene expression, tandem duplications occur at very high spontaneous frequencies and are easily reversible in that they may be readily lost by recombination when selective conditions change. It is tempting to speculate that duplications might occur in natural populations of bacteria under conditions where a crucial nutrient is limiting for growth. This idea is supported by the observation that chemostat cultures of *E. coli* and *Klebsiella aerogenes* growing on poor carbon sources become overgrown with duplications in the dissimilitory pathway for that carbon source (HORIUCHI, HORIUCHI and NOVICK 1963; RIGBY, BURLEIGH and HARTLEY 1974).

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LITERATURE CITED

CLARK, A. J., 1971 Toward a metabolic interpretation of genetic recombination. Ann. Rev. Microbiol. **25**: 437–464.

FOLK, W. R. and P. BERG, 1971 Duplication of the structural gene for glycyl-transfer RNA synthetase in *Escherichia coli*. J. Mol. Biol. **58**: 595–610.

FRANKLIN, N. C., 1971 Illegitimate recombination. pp. 175–194. In: *The Bacteriophage Lambda*. Edited by A. D. HERSHEY. Cold Springs Harbor Laboratory, New York.

GLANSDORFF, N. and G. SAND, 1968 Duplication of a gene belonging to an arginine operon of *Escherichi coli* K-12. Genetics **60**: 257–268.

GUTNICK, D., J. M. CALVO, T. KLOPOTOWSKI and B. N. AMES, 1969 Compounds which serve as the sole source of carbon or nitrogen for *Salmonella typhimurium* LT-2. J. Bacteriol. **100**: 215–219.

HILL, C. W., J. FOULDS, L. SOLL and P. BERG, 1969 Instability of a missense suppressor resulting from a duplication of genetic material. J. Mol. Biol. **39**: 562–581.

HILL, C. W. and G. COMBRIATO, 1973 Genetic duplications induced at very high frequency by ultraviolet irradiation in *Escherichia coli*. Mol. Gen. Genet. **127**: 197–214.

HORIUCHI, T., S. HORIUCHI and A. NOVICK, 1963 The genetic basis of hypersynthesis of β-galactosidase. Genetics **48**: 157–169.

KAY, W. and H. KORNBERG, 1971 Uptake of C_4-dicarboxylic acids by *Escherichia coli*. Eur. J. Biochem. **18**: 274–281.

LO, T. C. Y., M. K. RAYMAN and B. D. SANWAL, 1972 Transport of succinate in *Escherichia coli* I. Biochemical and genetic studies of transport in whole cells. J. Biol. Chem. **247**: 6323–6331.

MANDELSTAM, J. and K. MCQUILLEN, 1968 *Biochemistry of Bacterial Growth*. John Wiley and Sons Inc., New York.

OHNO, S., 1970 *Evolution by Gene Duplication*. Springer-Verlag, New York.

RIGBY, P. W. J., B. D. BURLEIGH and B. S. HARTLEY, 1974 Gene duplication in experimental enzyme evolution. Nature **251**: 200–204.

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.

SANDERSON, K. E., 1972 Linkage map of *Salmonella typhimurium*, Edition IV. Bacteriol Rev. **36**: 558–586.

SMITH, H. O. and M. LEVINE, 1967 A phage P22 gene controlling integration of prophage. Virology **31**: 207–216.

VOGEL, H. J. and D. M. BONNER, 1956 Acetylornithinase of *E. coli*: a partial purification and some properties. J. Biol. Chem. **218**: 97–106.

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