J. Mol. Biol. (1978) **119**, 147–166

Tandem Chromosomal Duplications in *Salmonella typhimurium*: Fusion of Histidine Genes to Novel Promoters

R. Philip Anderson† and John R. Roth

Department of Biology
University of Utah
Salt Lake City, Utah 84112, U.S.A.

(Received 16 August 1977)

Salmonella strains harboring tandem chromosomal duplications have been identified following selection for expression of a histidine biosynthetic gene whose promoter is deleted. In such strains, tandem duplications fuse the selected *his* gene to "foreign" regulatory elements, thereby allowing gene expression. Selection is made for *his*D$^+$ activity in deletion strain *his*OG203. Among the revertants, strains harboring tandem chromosomal duplications have been identified by a number of their properties. (1) Their HisD$^+$ phenotype is genetically unstable. (2) Such instability is dependent on recombination (*rec*A) activity. (3) Genetic tests demonstrate that these strains are merodiploid for large regions (up to 25%) of the *Salmonella* genome. (4) Recipient strains that inherit the HisD$^+$ phenotype of these duplication-carrying revertants also inherit the donor's merodiploid state. (5) In certain revertants the functional *his*D$^+$ gene and the sequence which promotes merodiploid transductant formation are linked to chromosomal markers located far from the normal *his* region.

Previous reports have concluded that the instability of strains isolated by this selection is due to translocation of the *his*D$^+$ gene to an extrachromosomal element (the *pi-histidine* factor). We believe that in all strains we have tested (33 independent isolates) instability can better be accounted for as due to tandem duplication events which permit expression of *his*D. At least two mechanisms are responsible for duplication formation. One mechanism is dependent on recombination function and generates identical revertants having a duplication of 16% of the chromosome. A second mechanism operates independently of recombination activity; individual duplications produced by this process have variable endpoints.

1. Introduction

Duplication of genetic material has been suggested to be of importance in molecular evolution (Hegeman & Rosenberg, 1970; Ohno, 1970). Duplications may increase the gene dosage of a required allele, provided fixed heterozygosity of polymorphic variants, or supply the redundant DNAs needed for genetic divergence. Within the past several years methods have been developed for the detection and analysis of tandem genetic duplications in bacteria and their phages. The literature on this work has recently been reviewed (Anderson & Roth, 1977*a*). Selections designed to detect cells harboring tandem duplications have generally been based on either the increased gene dosage that the duplication event confers, the heterozygosity that the merodiploid condition allows, or properties of the novel base sequence that is located at the join point between tandemly duplicated regions. This base sequence (often termed the

† Present address: Medical Research Council Laboratory of Molecular Biology, Hills Road, Cambridge, CB2 2QH, England.

novel joint; Hershey, 1970) is not found in the haploid chromosome from which a tandem duplication is derived. Under appropriate conditions, this novel base sequence may confer a selectable phenotype; cells harboring a duplication may then be detected. Detection methods of this type most frequently involve selection for turn-on of genes whose expression has been prevented by either polarity effects or inactivation of promoter elements. Tandem duplications may serve to fuse intact, unexpressed structural genes to functional promoters. Thus, the novel base sequence results from the juxtaposition of a functioning promoter and the structural gene whose expression is selected. This novel sequence is quite analogous to those formed when fusion of operons is achieved by the deletion of intervening material (Miller *et al.*, 1970).

Selections of this general nature have detected in *Escherichia coli* tandem duplications of the *arg*ECBH bipolar operon (Glansdorff & Sand, 1968; Elseviers *et al.*, 1969,1972; Cunin *et al.*, 1970), of the *trp* operon (Jackson & Yanofsky, 1973), of the *gal*ETK operon (Hill & Echols, 1966; Morse, 1967; Ahmed, 1975), of the tryptophanase gene (Yudkin, 1977), of the bacteriophage T4*r*IIB cistron (Freedman & Brenner, 1972), and of the bacteriophage P2 early genes *A* and *B* (Chattoraj & Inman, 1974; Bertani & Bertani, 1974). In each case, tandem duplications cause constitutive expression of the selected gene(s). The amount of duplicated material was generally found to be rather small (1 to 100 genes). Identification of tandem duplications as being responsible for the selected phenotypes has largely been the result of genetic analysis. The most frequently used criteria for identification of tandem duplications are merodiploidy for nearby genetic markers and genetic instability that is dependent on recombination function. In the case of bacteriophage P2, DNA–DNA heteroduplex analysis has provided direct physical evidence for the tandem duplication event (Chattoraj & Inman, 1974).

Ames *et al.* (1963) have described a selection in the histidine operon of *Salmonella typhimurium* very similar to those outlined above. Genetically unstable strains may be obtained by selecting for expression of *his* gene products whose synthesis has been prevented by deletion of the *his* operator–promoter region. Deletion *his*OG203 removes the *his* operator–promoter and a portion of the *his*G structural gene (see Fig. 1). It leaves the remaining *his* genes intact but unexpressed. Expression of HisD$^+$ activity may be selected as the ability to utilize the intermediate histidinol as a source of histidine. Among the HisD$^+$ revertants of *his*-203 is a class which is highly unstable for its selected phenotype. When such HisD$^+$ clones are grown nonselectively, HisD$^-$ segregants accumulate at a high frequency; these segregants are identical to the parental deletion mutant, *his*-203. In the original description of this phenomenon (Ames *et al.*, 1963) and in subsequent investigation (Levinthal & Yeh, 1972), the instability of these strains was interpreted as evidence that the functional

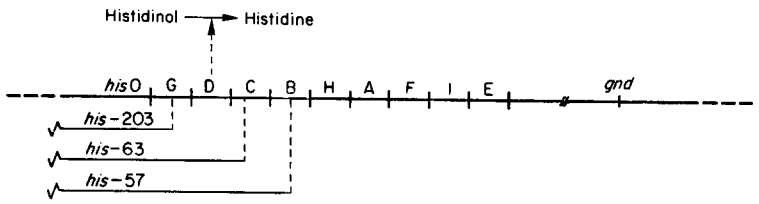

FIG. 1. A genetic map of the histidine operon. Deletion *his*-203 exhibits no detectable expression of *his*D and *his*C enzymes. The remaining genes (*his*B through *his*E) are weakly expressed by the low-level constitutive promoter *P2*, located between *his*C and *his*B (Atkins & Loper, 1970).

*his*D$^+$ gene had been translocated to an extrachromosomal plasmid element (termed the *pi-histidine* factor). The *pi-his* factor was considered to replicate autonomously. and instability was thought to result from unequal segregation of this plasmid.

Numerous cases of genetic instability in both *Salmonella* and *E. coli* have been attributed to the occurrence of tandem chromosomal duplications in these organisms. It occurred to us that many of the characteristics exhibited by *pi-his* revertants are also exhibited by strains harboring known tandem duplications of the histidine operon. Such duplications have been isolated following generalized transductional crosses that enrich for complementing heterogenotes (Anderson *et al.*, 1976). In this paper we propose that *pi-his* revertants of deletion *his*-203 contain tandem chromosomal duplications which fuse the duplicated *his*D gene to functional promoter elements. that this structure provides for expression of the *his*D$^+$ gene, and that loss of HisD$^+$ activity results from homologous recombination between the two copies of duplicated material; such recombination events excise intervening material and result in loss of the functional *his*D$^+$ gene.

The genetic characteristics of *pi-his* revertants (instability, merodiploidy, and transducibility of the merodiploid condition) are standard behavior of tandem chromosomal duplications. Two classes of *pi-his* duplications have been found. One class is formed by a recombination-dependent process; independent isolates of this type are duplicated for an identical 16% of the *Salmonella* chromosome. A second, heterogeneous class is formed by a recombination-independent mechanism; individual isolates are duplicated for various amounts of nearby material.

A preliminary account of this work has been presented elsewhere (Anderson & Roth, 1977*b*).

2. Materials and Methods

(a) *Media and growth conditions*

Vogel & Bonner (1956) E medium containing 2% glucose was used as minimal medium. When required, this medium was supplemented with 0·1 mM (excess) or 0·005 mM- (limiting) histidine, 2·0 mM-histidinol, 0·4 mM each of adenine, guanine and arginine, 0·05 mM-thiamin, and approx. 0·3 mM-other amino acids. DL amino acids were often used, but the concentrations given are for the L isomer. 3-Amino-1,2,4-triazole (Aldrich) was added at 20 mM final concn. When desired as a sole carbon source, D-sorbitol (Sigma) was added at 0·2% to E medium from which glucose and citrate had been omitted. Difco nutrient broth (0·8%) containing 0·5% NaCl was used as complex medium. Solid media contained 1·5% Difco agar. All incubations were at 37°C. Liquid cultures were aerated by gyrotory shaking.

(b) *Bacterial strains*

The genotypes and sources of selected strains used in this study are shown in Table 1. Strains with TT designations are those in our collection that either contain or are descended from a strain containing a copy of the translocatable tetracycline-resistance determinant Tn*10* (Kleckner *et al.*, 1975). All strains are derivatives of *S. typhimurium* strain LT2.

Strain TR4178 (*his*-203 *srl*-201) was isolated following diethyl sulfate mutagenesis of *his*-203 and 2 cycles of penicillin enrichment for Srl$^-$ (sorbitol non-utilizing) clones. Both mutagenesis and pencillin enrichment were performed according to the procedures of Roth (1970). The mutation *srl*-201 is approx. 50% linked to *rec*Al by P22-mediated generalized transduction. The close proximity of these two loci in *E. coli* has been described (McEntee, 1976; A. J. Clark, personal communication). Strain TR4192 (*his*-203 *srl*-201 *rec*Al *str*A) was constructed by mating strain TR4178 with donor strain TR2246 (*met*A22 *rec*Al *str*A HfrB2). Streptomycin-resistant conjugants were selected and the desired *srl*$^-$ *rec*A$^-$ recombinant was identified among the progeny. Strain TR2951 (*his*-63

TABLE 1

List of strains

Strain	Genotype	Source
his-203	*his*OG203	P. E. Hartman
his-63	*his*OGDC63	P. E. Hartman
TR2246	*met*A22 *rec*Al *str*A HfrB2	J. Wyche
TR2951	*his*-63 *rec*Al *str*A	TR2246 × *his*-63
TR4178	*his*-203 *srl*-201	This paper
TR4192	*his*-203 *srl*-201 *rec*Al *str*A	TR2246 × TR4178
PS29	*his*-57 (*pi*-2)	C. Gritzmacher
TT14	*met*C1975::Tn*10*	This paper
TT126	*tyr*A555::Tn*10*	This paper
TT142	*arg*E1828::Tn*10*	This paper
TT146	*arg*B1832::Tn*10*	This paper
TT169	*ser*A977::Tn*10*	This paper
TT172	*cys*G1510::Tn*10*	This paper
TT173	*cys*C1511::Tn*10*	This paper
TT215	*lys*A565::Tn*10*	This paper
TT233	*met*F877::Tn*10*	This paper
TT278	*gua*A554::Tn*10*	This paper
TT287	*pur*C882::Tn*10*	This paper
TT315	*pur*G1739::Tn*10*	This paper
TT317	*pur*F1741::Tn*10*	This paper
TT418	*gly*A540::Tn*10*	This paper
TT744	*his*-63 *arg*B1832::Tn*10*	TT146 × *his*-63
TT1720	*aro*D5 *zhf*-105::Tn*10*	G. Ames
TT1738	*aro*D5 *his*W1824 *met*G319 *pur*F145 *str*A *his*-63 *zgf*-2::Tn*10*	This paper
NK186	*cys*A1539::Tn*10*	N. Kleckner

All strains are derivatives of *S. typhimurium* strain LT2. See Materials and Methods for the derivation of strains original to this paper.

*rec*Al *str*A) was constructed by a similar cross using *his*-63 as the F^- recipient. Recombination deficiency (*recA*$^-$) was scored as sensitivity to approximately 400 ergs/mm^2 of ultraviolet light irradiation. Strain PS29 (*his*-57 [*pi*-2]) harbors the reversion event *pi*-2 originally described by Ames *et al.* (1963). PS29 was used as our source of *pi*-2 and was kindly supplied by C. Gritzmacher.

A large number of nutritional auxotrophs resulting from insertion of the Tn*10* element (Kleckner *et al.*, 1975) have been isolated in our laboratory as a co-operative effort. The sites of insertion have been identified in many of these auxotrophs by as many as 3 independent tests: (1) the ability of selected biosynthetic intermediates to fulfil nutritional requirements (crystal tests); (2) a demonstration of transductional linkage of Tn*10* insertions to known genetic markers; and (3) the marked reduction in numbers of prototrophic recombinants obtained when Tn*10* insertions are crossed with allelic mutations of known genotype. The results of these tests have led to the unambiguous assignment of many Tn*10* insertions to defined genes. Identification of the insertion site in TT317 (purF1741::Tn*10*) was made by J. Gots (personal communication).

Strain TT1738 (*aro*D5 *his*W1824 *met*G319 *pur*F145 *str*A *his*-63 *zgf*-2::Tn*10*) was derived from strain SB562 (*aro*D5 *his*W1824 *met*G319 *pur*F145 *str*A [P22]) in a manner designed to eliminate the P22 prophage present in SB562. Phage grown on TT184 (*pro*A622::Tn*10*) was used to transduce SB562 selecting tetracycline resistance. Since *pro*AB and *ata*A (the P22 prophage attachment site) are transductionally linked, many of the $Tet^R Pro^-$ recombinants had recombined out the prophage. One recombinant of this type (TT1723) was transduced to Pro^+ with phage grown on LT2. The resulting $Pro^+ Tet^S$ recombinant (TT1727) was then used as a recipient for donor strain TT1721 (*his*-63 *zgf*-2::Tn*10*). This donor contains

a Tn*10* insertion (isolated by F. Chumley) in "silent" DNA approx. 90% linked to the deletion *his*-63. Using this donor, most Tet^R transductants inherited *his*-63 in addition to the Tn*10* element. One such recombinant is strain TT1738.

(c) *Genetic techniques*

(i) *Isolation of* pi-his *revertants*

In order to measure the frequency of $HisD^+$ reversion of selected strains, nutrient broth grown cells were collected by centrifugation, washed with a minimal salts solution, and concentrated 10-fold. Samples were spread on E minimal plus 2·0 mM-histidinol plates. After 3 days incubation, the numbers of $HisD^+$ colonies were scored. For the isolation and analysis of independent *pi-his* revertants, individual nutrient broth cultures were inoculated with single colonies of either strain TR4178 (*his*-203 *srl*-201) or TR4192 (*his*-203 *srl*-201 *rec*Al *str*A). Following overnight growth, portions were spread individually onto E minimal plus 2·0 mM-histidinol plates. From each culture, 1 revertant was picked, purified, and analyzed further. Unstable isolates were assigned *pi-his* allele numbers; *pi*-401 through *pi*-421 were isolated from TR4178 ($recA^+$); *pi*-422 through *pi*-432 were isolated from TR4192 ($recA^-$).

(ii) *Visualization of* $HisD^-$ *segregants*

In order to test the genetic stability of the $HisD^+$ phenotype of various strains, single colonies of these strains were picked from selective plates (E minimal plus histidinol) and allowed to grow for 15 to 20 generations non-selectively in liquid nutrient broth. These cultures were then diluted and single colonies were spread on minimal plates containing both histidinol (2 mM) and a limiting concentration of histidine (0·005 mM). Under these conditions, $HisD^+$ clones form large round colonies, while $HisD^-$ clones form small, flat, easily distinguishable colonies.

(iii) *Transductions*

A derivative of the high-transducing phage P22 HT105/1 (Schmieger, 1971) was used in all transductions. This derivative (P22 HT105/1 *int*-201) was obtained in our laboratory by G. Roberts following hydroxylamine mutagenesis of P22 HT105/1 and a screen for non-lysogenizing variants. For transductions, plates were spread with a mixture of 2×10^8 to 6×10^8 recipient cells and approx. 10^8 phage particles. Transductant clones were scored after 2 to 4 days incubation at 37°C. When transductants were to be used in further work, clones were purified 3 times selectively, verified to be free of phage, and preserved.

(iv) *Preservation of unstable strains*

Cultures of unstable duplication strains in liquid medium were supplemented to contain 8% dimethyl sulfoxide and frozen at −70°C. We have found this technique exceptionally good for long-term preservation of strains whose desired genetic determinants are unstable (duplications, F′ episomes, Hfr strains, etc.).

3. Results

(a) *The genetic consequences of tandem duplications*

Before presenting specific models or experimental details, it is important to first consider several formal genetic aspects of tandem duplications.

(i) *Tandem duplications generally cause no loss of function*

The reasons why tandem duplications are generally non-destructive can be seen in the diagram of a tandem duplication presented in Figure 2. In the chromosome carrying the duplication, the only impropriety in base sequence is located at the marked point between the two tandemly repeated copies (*i-d*). This impropriety does

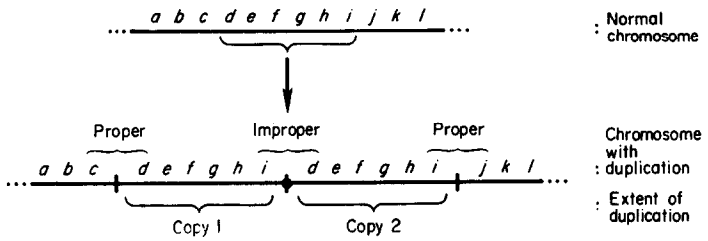

FIG. 2. Tandem duplication of the chromosomal segment *defghi*. Lower-case letters are non-genetic indications of hypothetical base sequences.

not lead to a loss of function, because proper versions of these sequences are present elsewhere (*c-d*, at the left; *i-j*, at the right). Thus, large tandem duplications do not destroy any genetic information. Exceptions to this rule are encountered when both endpoints of a duplication are located within a single gene or operon.

(ii) *Tandem duplications may be of unlimited size*

Since duplications lead to no loss of function, they are unlikely to be deleterious. Thus, even very large duplications may be maintained. The only restriction on the permissible size of a duplication might be the ability of the cell to replicate and segregate this large chromosome faithfully.

(iii) *Tandem duplications are subject to frequent loss*

Tandem duplications are unstable genetic structures. Reciprocal recombination between the two copies of duplicated material serves to excise intervening DNA and yields haploid chromosomes. Since this process involves legitimate recombination between homologous sequences, it might be expected to occur frequently. Loss of the duplication should depend strongly on recombination.

(iv) *Extremely large tandem duplications can be transduced*

As mentioned earlier, the only novel base sequence in chromosomes carrying a tandem duplication is located at the join point between copies of the duplicated region. At this point, sequences are made contiguous that would be widely separated in a normal chromosome. Transduction of this join point into a normal (haploid) recipient can serve to re-establish the donor's duplication state in recipient cells. This is possible even when the region included in the duplication is much too large to be carried by a single transducing fragment. Recombination events which account for this behavior are depicted in Figure 3. A transducing fragment that carries the join point of a tandem duplication contains base sequence homology to two widely separated regions on recipient chromosomes. When such a fragment enters recipient cells, reciprocal recombination events between the fragment and two recipient chromosomes regenerate the donor's duplication state. In the resulting recombinant, most of the duplicated material is derived from the recipient chromosome; only material immediately adjacent to the join point is derived from the donor. Thus, transduction of large tandem duplications may be detected, provided the selected donor marker and the join point between duplicated material are cotransducible. Transductional events such as those described in Figure 3 were first suggested by

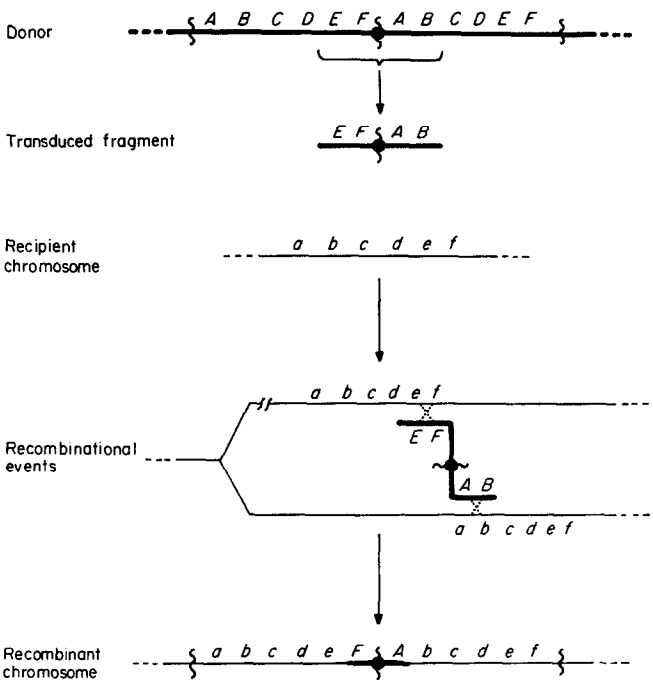

FIG. 3. A mechanism for transduction of large tandem duplications (Campbell, 1965; Hill *et al.*, 1969). Recipient and donor DNA are light and bold lined, respectively. Dotted lines represent reciprocal recombination events.

Campbell (1965). Strong genetic evidence in support of these events was subsequently presented (Hill *et al.*, 1969).

(b) *Isolation and behavior of* pi-his *revertants*

Deletion *his*0G203 removes the operator–promoter and a portion of the first structural gene of the histidine operon (see Fig. 1). The *his*D gene in strain *his*-203 remains structurally intact, but unexpressed. Three classes of HisD$^+$ (histidinol utilizing) revertants of *his*-203 have been described (Ames *et al.*, 1963; St. Pierre, 1968): (1) deletion mutations that extend the deleted region and fuse the *his*D gene to nearby constitutive promoters; (2) point mutations within the residual *his*G gene that provide a new promoter for *his*D expression; and (3) unstable mutations that "revert" at a high frequency to their parental genotype. Such unstable mutations have been designated *pi-his* revertants, due to the presumed translocation of the functioning *his* genes to an extrachromosomal element (the *pi-his* factor).

In the original description of this phenomenon, Ames *et al.* (1963) demonstrated that: (1) HisD$^-$ segregants that arise from unstable *pi-his* revertants are identical to the parental deletion *his*-203; (2) recipient strains that have inherited the HisD$^+$ phenotype of *pi-his* revertants are themselves genetically unstable; (3) all *his* genes (except *his*G) are constitutively expressed in *pi-his* revertants; and (4) the functional *his*D$^+$ gene in *pi-his* revertants is not transductionally linked to the normal *his* operon.

We propose a model for the structure of *pi-his* revertants based on tandem duplication of chromosomal material.

(c) *A proposed structure for* pi-his *revertants*

Our model to account for the structure of *pi-his* revertants is shown in Figure 4. There are promoters (termed *P'*) located at some distance from the histidine operon which have the same direction of transcription as does the histidine operon. Tandem duplication of material from a point within the *his* operon to a point within the transcription unit *P'* leads to fusion of the duplicated histidine genes to these "foreign" regulatory elements. In the resulting structure, the duplicated *his*D$^+$ gene is expressed under control of the promoter *P'*, replicated as a part of the chromosome, lost as a result of recombination between copies of duplicated material, and transduced by the mechanism outlined in Figure 3 (see above). The non-destructive nature of tandem duplications suggests that the amount of duplicated material might be quite large. In the following two sections we shall present evidence which confirms important predictions of this model. In later sections the mechanisms of duplication will be considered. We should like to distinguish between two types of duplications: those formed by recombination-dependent and recombination-independent processes.

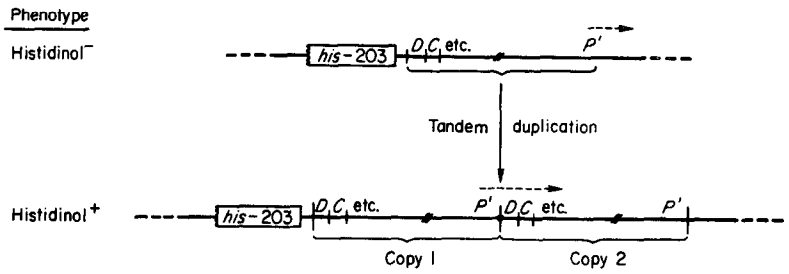

FIG. 4. A proposed structure for *pi-his* revertants. The broken arrows indicate the direction of transcription from promoter *P'*.

(d) *Instability of* pi-his *revertants is dependent on recombination*

Tandem genetic duplications generate haploid segregants as a result of homologous recombination between duplicated regions (Campbell, 1963). Thus, if the instability of *pi-his* is due to a tandem duplication, this instability should be evident only in recombination-proficient backgrounds. The crosses shown in Figure 5 demonstrate that this is true for *pi-his* revertants. *rec*A$^-$ alleles were introduced into *pi-his* revertants by use of the transductionally linked marker *srl*$^-$. Srl$^-$ strains are unable to utilize D-sorbitol as a sole carbon source. In the first cross shown in Figure 5, a *srl*$^+$*rec*A$^-$ donor has been used to transduce a number of independent *srl*$^-$*rec*A$^+$*pi-his* revertants, and the isogenic Rec$^+$ and Rec$^-$ recombinants were identified among the progeny. Rec$^+$ recombinants exhibit the same high frequency of HisD$^-$ segregants characteristic of *pi-his* revertants (average = 35% segregants following 15 to 20 generations of non-selective growth for the six strains tested). Rec$^-$ recombinants exhibit no HisD$^-$ segregants (<0·02%). Yet, these stable strains still contain the *pi-his* structure; when they are used as donors in crosses designed to recover *pi-his* from them (cross no. 2), the characteristic instability reappears in the resulting *rec*A$^+$*pi-his* recombinants. Stability of *pi-his* in *rec*A$^-$ backgrounds has been demonstrated for five independent *pi-his* revertants isolated by us (*pi*-404, -413, -414, -420, and -421) and for the revertant *pi*-2 described by Ames *et al.* (1963). Based on these

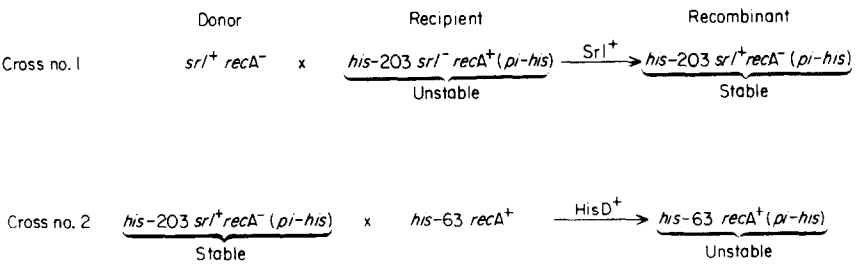

FIG. 5. Transductional crosses demonstrating that instability of *pi-his* revertants is dependent on recombination. Selected phenotypes are indicated above the arrows. Multiply marked strains used were TR2951 (*his*-63 *srl*$^+$ *rec*Al *str*A) and 6 independent *pi-his* revertants (*pi*-404, *pi*-413, *pi*-414, *pi*-420, *pi*-421 and *pi*-2). The revertant *pi*-2 is described by Ames *et al.* (1963). Mutation *his*-63 is a large deletion whose map position is depicted in Fig. 1. All crosses were performed as described in Materials and Methods.

experiments, we conclude that *pi-his* revertants are at least 1700-fold more stable in recombination-deficient backgrounds.

(e) pi-his *revertants are merodiploid for large chromosomal regions*

By a variety of genetic techniques, we have determined the amount of duplicated material in 33 independent *pi-his* revertants. Our method of isolating *pi-his* revertants guarantees them to be of independent origin (see Materials and Methods). Of these revertants, 22 were isolated in a *rec*A$^+$ genetic background; the merodiploid content of these strains is presented in this section. The remainder were isolated in a *rec*A$^-$ background; the results of these tests are presented in a later section (see below) that describes the role of recombination in *pi-his* formation.

The basic scheme for determining the extent of duplicated material in *pi-his* revertants has been to determine whether they can be made heterozygous for nearby genetic markers. If they can, then these strains must be merodiploid for those markers. Three different techniques have been used to determine the merodiploidy of nearby markers. In most cases, merodiploidy has been tested through the use of auxotrophic mutations generated by insertion of the transposable tetracycline-resistance determinant Tn*10* (Kleckner *et al.*, 1975). These auxotrophs result from the linear insertion of the Tn*10* element into defined structural genes. The Tn*10* element specifies a selectable phenotype (tetracycline resistance), as well as causing the auxotrophy phenotype. Selection for inheritance of Tn*10* (by selecting tetracycline resistance) demands that recipients also inherit the lesion caused by Tn*10* insertion. When haploid strains inherit such Tn*10* insertions, they inherit the donor's auxotrophy as well. However, if the recipient strain is merodiploid for the Tn*10* insertion site, tetracycline-resistant recombinants remain prototrophic, due to the presence of a second (wild-type) copy of the gene involved and the recessive nature of these mutations. Transductional crosses of this type allow rapid testing of whether a given strain is merodiploid for known Tn*10* insertion sites. When Tn*10* insertions were not available for markers to be tested, alternative procedures were used. For testing of *met*G and *srl*, *pi-his* revertants were either isolated in or transduced into genetically marked backgrounds (*met*G$^-$ or *srl*$^-$). If such strains were merodiploid for *met*G$^-$ or *srl*$^-$, subsequent transductions (to MetG$^+$ or Srl$^+$) yielded clones that were heterozygous. Analysis of the genotypes of the *his*D$^-$ haploid segregant population from these clones

then revealed any heterozygosity. That is, if both *met*G$^+$ and *met*G$^-$ (or *srl*$^+$ and *srl*$^-$) clones were present among the HisD$^-$ segregants, the strain from which these segregants arose must have been heterozygous for the *met*G (or *srl*) region. Merodiploidy for *rec*A was similarly tested by transducing *rec*A$^-$ alleles into *pi-his* revertants using the linked marker *srl*$^-$. These heterozygotes were then further analyzed by screening the HisD$^-$ segregants for the *rec*A$^-$ phenotype. (*rec*A$^+$/*rec*A$^-$ heterozygotes are phenotypically Rec$^+$ and therefore unstable.) Merodiploidy of *aro*D was tested by use of strain TT1720 (*aro*D5 *zhf*-105::Tn*10*). This strain harbors a Tn*10* insertion (isolated by G. Ames) approximately 40% linked to the mutation *aro*D5. When haploid recipients are transduced with this donor, 40% of Tet^R recombinants inherit *aro*D5. When recipients merodiploid for the *aro*D$^+$ gene are used, no apparent linkage is observed (*aro*D5 is recessive).

In Table 2 the results of these tests are presented for *pi-his* revertants that formed in a *rec*A$^+$ background. Among 22 independent *pi-his* revertants investigated, three classes are revealed. Class I isolates are merodiploid for the nearby locus *met*G. They are not merodiploid for the next tested marker, *pur*F. Thus, *pi-his* revertants of this type are merodiploid for a chromosomal segment that is 3% to 8% of the *Salmonella* genome. Class I isolates total 6 of the 22 independent revertants and include the isolate *pi*-2 described by Ames *et al.* (1963). Class II revertants form the majority (15 of 22 isolates). They are merodiploid for all loci tested (as many as 12) in the region from *his* through *arg*B. Thus, class II revertants harbor a merodiploid region of approximately 16% of the genome. A single revertant (class III) is merodiploid for each of 17 loci tested in the region from *his* through *arg*E; this strain is merodiploid for approximately 25% of the *Salmonella* genome.

It is very important to note that the results presented in Table 2 were obtained with strains that had inherited by transduction the HisD$^+$ phenotype of the original *pi-his* revertants. Thus, transduction of the HisD$^+$ phenotype into new recipients establishes a merodiploid state in these *pi-his* recombinants. This merodiploid state may even be as large as 25% of the genome. Considering the transducible nature of large tandem genetic duplications (Hill *et al.*, 1969; see Fig. 3), we interpret these results as evidence that the functional *his*D$^+$ gene in *pi-his* revertants is located near the join point of a tandem chromosomal duplication.

For *pi-his* revertants of the class II type, the location of the duplication join point and the linkage of a functional *his*D$^+$ gene to this join point may be demonstrated quite dramatically. Class II revertants are merodiploid for all loci tested in the region from *his* through *arg*B (see Table 2). They are not merodiploid for the gene *lys*A. *arg*B and *lys*A are approximately one minute separated on the *Salmonella* genetic map. The functional *his*D$^+$ gene in class II revertants may be shown to be linked to the *arg*B gene by the following experiment: when any class II revertant is used as a transductional donor and an *arg*B$^-$*his*D$^-$ strain is used as a recipient, approximately 33% of Arg$^+$ transductants inherit the HisD$^+$ phenotype non-selectively. These data are shown in Table 3. The resulting Arg$^+$HisD$^+$ recombinants are unstable for both their Arg$^+$ and their HisD phenotypes. Moreover, they are merodiploid for the entire chromosomal region from *his* through *arg*B. We presume that these transductants arise as diagrammed in Figure 3 and interpret these results as indicating cotransduction between the *arg*B gene, the join point of a tandem duplication, and a functional *his*D$^+$ gene in class II *pi-his* revertants. Linkage data of this type are strong evidence for a tandem chromosomal duplication contained by *pi-his* revertants.

TABLE 2

Merodiploidy of independent rec*A*$^+$ pi-his *revertants*

Map position (min): Source of *pi-his*	Marker tested *met*G 46·8	*pur*F 49·6	*aro*D 49·9	*cys*A 51·7	*pur*C 52·8	*gua*A 53·6	*gly*A 54·4	*pur*G 54·7	*tyr*A 56·1	*rec*A 57·6	*srl* 57·7	*cys*C 58·8	*arg*B 59·9	*lys*A 60·8	*ser*A 62·3	*met*C 63·9	*arg*E 67·9	*cys*G 72·8	*met*F 87·2	Class
pi-404	+	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−			I
pi-413	+	−		−	−	−	−	−	−	−	−	−	−	−	−	−	−			I
pi-414	+	−		−	−	−	−	−	−	−	−	−	−	−	−	−	−			I
pi-420	+	−		−	−	−	−	−	−	−	−	−	−	−	−	−	−			I
pi-421	+	−		−	−	−	−	−	−	−	−	−	−	−	−	−	−			I
pi-2	+	−		−						−	−									I
pi-401	+		+	+	+	+	+	+	+	+	+	+	+	−	−	−	−			II
pi-402				+	+	+	+	+	+	+	+	−	+	−		−	−			II
pi-403				+	+	+	+	+	+	+	+	+	+	−	−	−	−			II
pi-406					+	+	+	+	+	+	+	+	+	−	−	−	−			II
pi-407										+	+		+							II
pi-408				+	+	−	+	+	+	+	−	+	+	−	−	−	−			II
pi-409				+	+	−	+	+	−	+	+	+	+	−	−		−			II
pi-410				+	+	+	+	+	+	−	+	+	+	−	−	−	−			II
pi-411				+	+	+	+	+	+	+	+	+	−	−	−					II
pi-412				+	+	+	+	+	+	−	+	+	+	−		−	−			II
pi-415				+	+	+	+	+	+	+	+	+	+	−	−	−	−			II
pi-416				+	+	+	+	+	+	+	+	+	+	−		−	−			II
pi-417					+	−	+	+	+	+	−	−	−	−	−					II
pi-418					+		+	−		+	+	+	−	−	−		−			II
pi-419				+	+	+	+	−			+		+	−	−	−				II
pi-405	+	+	+	+	+	+	+	+	+	+	−	−	+	+	+	+	+		−	III

Merodiploidy was tested as described in the text. *pi-his* strains tested were derived from the original revertants by transducing the HisD$^+$ phenotype into new recipients. Markers are listed along the top in the order of increasing distance from *his*. A + indicates that the *pi-his* strain was tested and found to be merodiploid for the marker shown; a − indicates that the *pi-his* strain was tested and found to be haploid; a blank indicates that the *pi-his* strain was not tested. Strains used for testing merodiploidy were TT14, TT126, TT142, TT146, TT169, TT172, TT173, TT215, TT233, TT278, TT287, TT315, TT317, TT418, TT1720, TT1738, NK186 and TR2951. See Table 1 for their genotypes. The map positions shown are those of the *E. coli* linkage map (Bachmann *et al.*, 1976), to which the new *Salmonella* map will be standardized (Sanderson & Hartman, 1978). The *his* operon is located at min 44·1. All crosses were performed as described in Materials and Methods.

TABLE 3

Cotransduction of HisD⁺ with arg*B in class II* pi-his *revertants*

Source of donor *pi-his*	Revertant class	Arg⁺HisD⁻ recombinants	Arg⁺HisD⁺ recombinants	% Cotransduction
pi-401	II	323	97	23
pi-402	II	67	33	33
pi-403	II	33	17	34
pi-406	II	37	22	37
pi-407	II	74	38	34
pi-408	II	75	25	25
pi-409	II	66	34	34
pi-410	II	81	39	33
pi-411	II	38	22	37
pi-412	II	73	38	34
pi-415	II	30	14	32
pi-416	II	43	17	28
pi-417	II	40	22	35
pi-418	II	66	34	34
pi-419	II	62	38	38
pi-404	I	100	0	<1
pi-405	III	100	0	<1
LT2	—	100	0	<1

The recipient strain is TT744 (*his*-63 *arg*B1832::Tn*10*).

Arg⁺ recombinants were selected on minimal medium plus histidine plates. Transductants were picked and subsequently scored for their HisD phenotype. In each case one representative Arg⁺HisD⁺ recombinant was purified and verified to be unstable for both Arg⁺ and HisD⁺. All crosses were performed as described in Materials and Methods.

(f) *Frequency of* pi-his *reversion*

HisD⁺ revertants of strain TR4178 (*his*-203 *srl*-201) are obtained spontaneously at a frequency of $1{\cdot}7\times10^{-9}$ per cell. Of 148 independent revertants, 29 were found to be genetically unstable and therefore classified as *pi-his* revertants. Thus, *pi-his* reversion occurs at a frequency of 3×10^{-10} per cell. The nature of the stable revertants has been described (Ames *et al.*, 1963; St. Pierre, 1968). HisD⁺ revertants of strain TR4192 (*his*-203 *srl*-201 *rec*A1 *str*A) are obtained spontaneously at a frequency of $1{\cdot}2\times10^{-9}$ per cell. As expected, all revertants obtained in the *rec*A⁻ background are stably HisD⁺. However, when *rec*A⁺ alleles are introduced into these revertant strains (using the linked *srl*⁻ mutation), approximately 5% (11 of 244 independent revertants) become unstable. Thus, in a recombination-deficient background, *pi-his* revertants are obtained at a frequency of 5×10^{-11} per cell; this figure is approximately sixfold less than in a *rec*A⁺ background. Since instability is only evident after introduction of the *rec*A⁺ allele, these results confirm the observation that recombination is required for *pi-his* segregation. The sixfold reduced *pi-his* reversion frequency in *rec*A⁻ backgrounds appears to be explained by the absence of isolates having their duplication endpoints in the *arg*-B*lys*A region. Such isolates are frequent among revertants in a *rec*A⁺ background and are absent among *rec*A⁻ isolates. Therefore, class II isolates appear to be formed by a recombination-dependent mechanism. This conclusion is based on a comparison of the extent of merodiploidy harbored by *pi-his* revertants obtained in *rec*A⁺ and *rec*A⁻ backgrounds. These data are presented in the following section.

(g) *Distribution of* pi-his *duplication endpoints formed in* rec*A*$^-$ *backgrounds*

The extent of the merodiploid region harbored by each of 11 independent *pi-his* revertants isolated in a *rec*A$^-$ background has been determined. *rec*A$^+$ alleles were transduced into 244 independent HisD$^+$ revertants of strain TR4192 (*his*-203 *srl*-201 *rec*Al *str*A), and 11 unstable *pi-his* isolates were identified. The HisD$^+$ phenotype of these strains was then transduced into new recipients (*his*-63); the resulting *pi-his* recombinants were used to determine the extent of merodiploidy of each isolate. The methods used for detecting merodiploidy were those described in section (e) above. The results of these tests are presented in Table 4. Among the 11 revertants, seven classes are present. The class I revertant is not merodiploid for *met*G, and thus contains no detectable merodiploidy. It likely harbors a short duplication whose endpoint is located between *his* and *met*G. Class II revertants are distinguished from class III revertants (both of which have endpoints in the *met*G–*pur*F region), by the fact that the join point between duplicated regions and the functional *his*D$^+$ gene in class II revertants are approximately 10% linked to *met*G319. Class III revertants do not exhibit such linkage. Classes IV to VII are each merodiploid to a different extent. The largest duplication (contained by *pi*-431) is approximately 12% of the genome. The functional *his*D$^+$ gene of each class IV isolate is approximately 4% linked to the mutation *aro*D5. Similarly, the *his*D$^+$ gene of *pi*-429 (class VI) is 12% linked to *pur*G1739::Tn*10*.

Conspicuously absent from the *pi-his* isolates formed in *rec*A$^-$ cells are those having endpoints in the *arg*B–*lys*A region (class II *rec*A$^+$ revertants). Thus, revertants of this type (a majority of isolates formed in *rec*A$^+$ cells) appear to be generated by a recombination-dependent process. The 11 *rec*A$^-$ revertants are heterogeneous with respect to the size of the merodiploid region. However, identical independent isolates are occasionally obtained. This contrasts sharply with the results obtained in a *rec*A$^+$ background. In that case, most of the isolates (15 of 22) have the same 16% of the chromosome duplicated. The remainder arise at a frequency comparable to those obtained in the *rec*A$^-$ background by recombination-independent mechanisms.

(h) *Duplicated material within the* his *operon*

By testing each *pi-his* revertant for merodiploidy of a series of nearby loci, it was possible to determine the position of one endpoint of the duplicated material (see above). The highly non-random distribution of these endpoints (in *rec*A$^+$ isolates) prompted us to map the location of the second endpoint, located near the *his*D gene. We sought to determine whether these endpoints exhibit a non-random distribution as well. Ames *et al.* (1963) demonstrated that *pi-his* revertants complement all *his* mutations except those in *his*G. Levinthal & Yeh (1972) presented evidence that the breakpoint within the operon for expression of *his*D is located at a unique site within the *his*G gene. We have determined precisely the location within the *his* operon of the duplication endpoint in each of our *pi-his* revertants (both *rec*A$^+$ and *rec*A$^-$ isolates). This task was made easier by the availability of a revised genetic map of the *his*G gene (Hoppe *et al.*, manuscript in preparation). A large number of deletions affecting *his*G has recently become available (Scott *et al.*, 1975; Ino *et al.*, 1975). These mutations have allowed very sensitive fine-structure mapping of the gene. The resolution of the new map is quite high, due to the use of a high-frequency generalized

TABLE 4

Merodiploidy of independent rec*A*⁻ pi-his *revertants*

Map position (min): Source of *pi-his*	Marker tested *met*G 46·8	*pur*F 49·6	*aro*D 49·9	*cys*A 51·7	*pur*C 52·8	*gua*A 53·6	*gly*A 54·4	*pur*G 54·7	*try*A 56·1	*rec*A 57·6	*srl* 57·7	*cys*C 58·8	*arg*B 59·9	*lys*A 60·8	Class
pi-430	–	–	–	–	–	–	–	–	–	–	–	–	–	–	I
pi-424	+	–	–	–	–	–	–	–	–	–	–	–	–	–	II†
pi-426	+	–	–	–	–	–	–	–	–	–	–	–	–	–	II†
pi-422	+	–	–	–	–	–	–	–	–	–	–	–	–	–	III
pi-427	+	–	–	–	–	–	–	–	–	–	–	–	–	–	III
pi-423		+	+	–	–	–	–	–	–	–	–	–	–	–	IV
pi-425		+	+	–	–		–	–	–	–	–	–			IV
pi-432		+	+	–	–	–	–	–	–	–	–	–	–	–	IV
pi-428		+	+	+	–	–	–	–		–	–	–			V
pi-429			+	+	+		+	+	–	–	–	–	–	–	VI
pi-431			+	+	+		+	+	+	–	–	–	–	–	VII

Merodiploidy was tested as described in the text. *pi-his* strains tested were derived from the original revertants by transducing the HisD⁺ phenotype into new recipients. Markers are listed along the top in the order of increasing distance from *his*. A + sign indicates that the *pi-his* strain was tested and found to be merodiploid for the marker shown; a – sign indicates that the *pi-his* strain was tested and found to be haploid; a blank indicates that the *pi-his* strain was not tested. Strains used for testing merodiploidy were TT126, TT146, TT173, TT215, TT278, TT287, TT315, TT317, TT418, TT1720, TT1738, NK186 and TR2951. See Table 1 for their genotypes. The map positions shown are those of the *E. coli* linkage mall (Bachmann *et al.*, 1976), to which the new *Salmonella* map will be standardized (Sanderson & Hartman, 1978). The *his* operon is located at min 44·1. All crosses were performed as described in Materials and Methods.

† Revertant class II is distinguished from class III because the HisD⁺ phenotype of class II revertants is approx. 10% linked to *met*G139.

transducing mutant of phage P22 (Schmieger, 1971). The current *his*G genetic map includes 80 deletions and 95 point mutations. The deletions define 41 intervals among which the point mutations are distributed.

The procedure for mapping the duplication endpoints within *his*G was as follows: each *pi-his* duplication was transduced into deletion mutant *his*-63, selecting HisD$^+$. Deletion *his*-63 removes the entire *his*G and *his*D genes. Since transduction of the HisD$^+$ phenotype of *pi-his* revertants involves transducing the join point of a tandem duplication (see above), any *his*G or *his*D sequences carried by *his*-63 (*pi-his*) recombinants must be located near the duplication join point. These *pi-his* strains were used as donors in crosses designed to determine the amount of *his* material present. Any *his*G or *his*D sequences carried by these strains must have been duplicated in the original *pi-his* revertant.

The inclusion of *his*G material was tested by the ability of *his*-63 (*pi-his*) donors to transduce *his*G$^-$ point or deletion mutants selecting His$^+$. The inclusion of *his*D sequences was tested by the ability of *his*-63 (*pi-his*) donors to transduce *his*D$^-$ deletion mutants selecting aminotriazole resistance on minimal medium. When *his*D$^-$ deletion mutants are transduced to His$^+$ with *pi-his* donors, two types of recombinants are possible. The first type is true *his*$^+$ recombinants between the recipient deletion and the donor *his*D$^+$ gene. The second type of recombinant arises by re-establishment of the *pi-his* duplication. Since *his*D$^-$ strains are *his*G$^+$, they can inherit the *pi-his* merodiploid condition to form complementing His$^+$ unstable recombinants (Ames *et al.*, 1963). In testing recombinants for mapping purposes, the second type of recombinant was eliminated by selecting only aminotriazole-resistant clones. The histidine analog 3-amino-1,2,4-triazole is a specific inhibitor of the *his*B enzyme (Hilton *et al.*, 1965). Strains are inhibited by aminotriazole if they are unable to derepress their *his*B enzyme levels. True *his*$^+$ recombinants are aminotriazole-resistant because they carry a completely normal histidine operon. *pi-his* merodiploid recombinants are all aminotriazole-sensitive, presumably because the constitutive promoter(s) to which the *his*D gene is fused provide insufficient expression of *his*B to allow escape from inhibition. The recipient *his*B region cannot provide resistance, since only polar *his*D$^-$ deletions were used as recipients; polarity effects prevented high levels of expression of the second *his*B copy.

The results of these experiments are shown in Figure 6. Several points merit attention. (1) Each *pi-his* isolate contains all detectable *his*D material. This result is not surprising, since *pi-his* strains are phenotypically HisD$^+$. However, the amino-terminal portion of the *his*D polypeptide is not required for enzymatic activity. Genetic experiments demonstrate that certain operator-proximal *his*D deletion mutations retain *his*D$^+$ activity (Ino *et al.*, 1975; J. Loper, personal communication). Based on protein sequencing studies, the non-essential region has been estimated to be approximately 80 residues in length (T. Kohno, personal communication). Therefore, it is conceivable that the duplication endpoint might have been located within the *his*D gene. (2) Every *pi-his* isolate obtained in a *rec*A$^+$ background contains no detectable *his*G material. All 22 revertants have the same duplication endpoint; this point is located in the region between the *his*G and *his*D genes. Included in these studies was the isolate *pi*-2 described by Ames *et al.* (1963) and Levinthal & Yeh (1972). Our mapping results differ from those obtained in the latter study. (3) The location of *rec*A$^-$ *pi-his* endpoints are somewhat heterogeneous. Certain *pi-his* duplications formed in *rec*A$^-$ strains have endpoints within the *his*G gene. However,

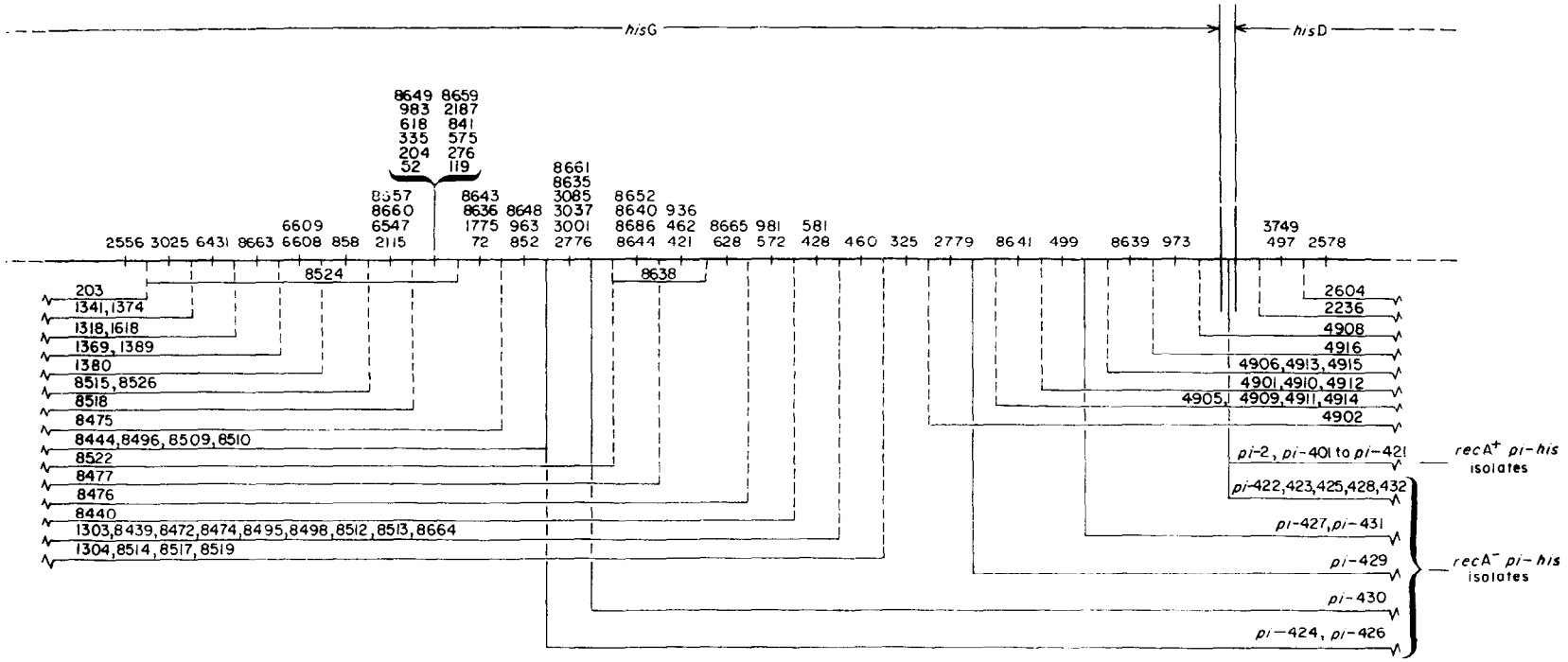

FIG. 6. The locations of *pi-his* duplication endpoints within the *his*G gene. The operator-distal portion of the *his*G genetic map (Hoppe *et. al.*, manuscript in preparation) is shown. *his*G and *his*D material carried by *pi-his* revertants was determined by transductional crosses using point or deletion mutants as recipients. All crosses were performed as described in Materials and Methods.

the *his*G–*his*D gene boundary again appears to be a preferred site for *pi-his* duplication formation.

4. Discussion

Among the HisD$^+$ revertants of deletion *his*-203 are strains that are highly unstable for their selected phenotype. In the original description of this phenomenon (Ames *et al.*, 1963) and in more recent work (Levinthal & Yeh, 1972) the genetic instability was interpreted as evidence that the functional *his*D$^+$ gene in these strains is attached to an extrachromosomal plasmid, termed the *pi-his* factor. However, numerous instances of genetic instability in both *E. coli* and *S. typhimurium* have been attributed to the occurrence of tandem genetic duplications in these organisms (for a review see Anderson & Roth, 1977*a*). It seemed to us that *pi-his* instability might be similarly explained. With this in mind, we have investigated the nature of *pi-his* revertants. The evidence presented above suggests that *pi-his* revertants harbor tandem chromosomal duplications which fuse the duplicated *his* genes to functional promoter elements. This structure (shown in Fig. 4) seems sufficient to account for all properties of *pi-his* revertants.

The experiments designed to detect merodiploidy of nearby genetic markers demonstrate that *pi-his* revertants harbor duplications of genetic material (see Tables 2 and 4). The duplications carried by *pi-his* revertants have been found to be quite large; individual isolates are duplicated for as much as 25% of the genome. Tandem duplications of equally large sections of the *Salmonella* chromosome have been reported (Straus & Hoffmann, 1975; Straus & Straus, 1976; Anderson *et al.*, 1976).

As predicted by the nature of tandem duplications, homologous recombination is required for both instability and inheritance of the merodiploid state of *pi-his* revertants. The stability of *pi-his* revertants in *rec*A$^-$ backgrounds (see Fig. 5) indicates that *pi-his* segregation occurs as the result of recombinational events, rather than the partitioning of plasmid molecules among progeny cells. This is strong evidence for a tandem chromosomal location of the duplicated copies. Inheritance of *pi-his* by transduction also occurs by a recombinational-dependent process (see Fig. 3). When the HisD$^+$ phenotype of *pi-his* revertants is transduced into *rec*A$^-$ recipients, no recombinants are obtained. These results are consistent with the mechanism for inheritance of tandem duplications outlined in Figure 3. In contrast, inheritance of *E. coli* R-factor plasmid molecules by P1-mediated transduction has been shown to be independent of *rec*A activity (Ohtsubo, 1970).

Treatment of *pi-his* revertants with agents that stimulate recombination (such as ultraviolet light) increases the rate of HisD$^-$ segregation (data not shown). The curing of *pi-his* merodiploidy by acriflavin (Levinthal & Yeh, 1972) has been interpreted as evidence for plasmids harbored by *pi-his* revertants. However, we have observed that a number of acridine compounds (including acriflavin) efficiently cure known tandem duplications (Anderson & Roth, manuscript in preparation). We suspect that these compounds increase the amount of recombination, possibly by inducing DNA repair systems (Witkins, 1976). This interpretation is supported by the observation that acriflavin curing of duplications depends on a functional recombination system. Since segregation of tandem duplications results from recombinational processes, increased recombination activity yields increased segregation.

Induced merodiploidy in *pi-his* recombinants (see Results, section (e)) and co-transduction of the HisD$^+$ unstable phenotype with distant chromosomal markers

(see Table 3 and Results, section (g)) are perhaps the strongest evidence in support of the tandem duplication model for *pi-his* formation. The requirements for detecting transduction of a tandem duplication (Campbell, 1965; Hill *et al.*, 1969) are most certainly met by the *pi-his* structure shown in Figure 4. The functional *his*D$^+$ gene (a selectable marker when used as a donor) is by necessity located near the join point of the tandem duplication, because the join point provides the promoter needed for *his*D$^+$ expression. Thus, recipient strains that inherit *pi-his* also inherit the characteristic merodiploidy. Quite often the *his*D$^+$ gene may also be shown to be linked to chromosomal markers unrelated to the *pi-his* selection. These markers are presumably located near the promoters to which the *his*D gene has been fused. This linkage is demonstrated for certain revertants by the observation that the three phenotypic properties of the duplication (HisD$^+$ expression, genetic instability, and chromosomal merodiploidy) can all be simultaneously co-inherited with a particular chromosomal region far from the *his* region. This non-selective inheritance of a region of merodiploidy is almost certainly the result of cotransducing the join point of a tandem duplication with the particular chromosomal region in question (see Fig. 3).

The most conservative interpretation of these results is that inheritance of *pi-his* occurs *at least initially* as a tandem chromosomal duplication. Any chromosome which harbors a tandem duplication can certainly generate covalently closed circular DNA as the result of reciprocal recombination between the two copies of duplicated material. Such molecules have been detected in strains harboring tandem duplications of the *E. coli gly*T locus (Hill *et al.*, 1977). In many respects, such molecules resemble plasmids. However, they should be lacking the gene(s) or site(s) necessary for autonomous replication. If such molecules can be replicated only by re-inserting into the chromosome, then it seems they may best be considered tandem chromosomal duplications. While we have no direct evidence precluding replication of this molecule, we feel that such replication is unnecessary to account for *pi-his* properties. A search for covalently closed circular DNA in *pi-his* revertants has been unsuccessful (H. Whitfield, personal communication).

Based on the frequencies and endpoints of *pi-his* duplications obtained in *rec*A$^+$ and *rec*A$^-$ backgrounds, we should like to distinguish between two duplication mechanisms. One mechanism is dependent on recombination function and is responsible for a majority (15 of 22) of the *pi-his* revertants obtained in a *rec*A$^+$ background. Such revertants (*rec*A$^+$ class II) are duplicated for the chromosomal region from *his* through *arg*B. The most attractive interpretation of these results is that there exists a DNA sequence in the *arg*B–*lys*A region which is partially or completely homologous to a sequence found within the *his* operon at the *his*G–*his*D border. Legitimate recombination between these sequences yields duplication (or lethal deletion) of intervening material. The remainder of the *rec*A$^+$ revertants (classes I and III) arise at a frequency comparable to *pi-his* revertants obtained in a *rec*A$^-$ background. Thus, these revertants arise either by recombination-independent processes (see below) or by low frequency, recombination-dependent events.

A second duplication mechanism occurs in the absence of recombination function. It, therefore, satisfies the definition of illegitimate recombination (Franklin, 1971). *pi-his* duplications generated by this mechanism are heterogeneous with respect to the amount of material duplicated. They occur at a frequency approximately sixfold less than recombination-dependent revertants. Duplications of the *E. coli arg*ECBH operon have been selected by a similar rationale, and have been reported to occur

independently of *rec*A function (Beeftinck *et al.*, 1974). In contrast, duplications of the *Salmonella trp* operon (selected as revertants of *trp* promoter mutations) are not found among revertants in *rec*A$^-$ strains; they are frequent among *rec*A$^+$ isolates (Basu & Margolin, 1972,1973; Margolin & Bauerle, 1966). These results likely reflect differences among strains in sequences available for unequal recombination with the gene under selection. If homologous sequences do not exist (or if they are not located within a functioning transcriptional unit), then only recombination-independent mechanisms are available.

pi-his tandem duplications are rare. They occur spontaneously at a frequency of approximately 3×10^{-10} per cell. Other tandem duplications in *Salmonella* have been estimated to be quite frequent (Miller & Roth, 1971; Straus & Hoffmann, 1975; Straus & Straus, 1976; Anderson *et al.*, 1976; Anderson & Roth, 1976). Estimates range from 4×10^{-5} to 2×10^{-1} per cell. Each of these estimates is based on selections which are relatively undemanding in terms of duplication endpoints; the duplications need only include the gene(s) under selection. The *pi-his* selection, however, is very restrictive of permissible endpoints. One endpoint must be within a small (500 to 1000 bases) region of the *his* operon; the other must be located within the transcription unit of a functioning and properly oriented promoter. Thus, only a small fraction of total duplications is selected.

The locations of *pi-his* duplication endpoints within the *his*G–*his*D region are remarkably non-random. Of 33 independent revertants, 27 have endpoints precisely at the *his*G–*his*D gene boundary. We estimate the target size of permissible endpoints in this region to be 500 to 1000 bases. Endpoints may fall anywhere within the operator-proximal ~240 base-pairs (~80 amino acids) of the *his*D gene, anywhere within the residual *his*G gene, or anywhere to the "left" of deletion *his*-203 such that no transcription termination sites are encountered (see Fig. 4). Yet most endpoints occur at the *his*G–*his*D boundary. This curious but unexplained observation may be related to the phenomenon of polarity (Franklin & Luria, 1961; Jacob & Monod, 1961). Certain duplication events having endpoints within *his*G would be expected to generate polar effects at the junction between duplicated regions. If the *his*D gene is fused to a low-level promoter, these polarity effects might reduce *his*D expression and prevent utilization of histidinol. Such duplications would not be recovered as *pi-his* revertants. Duplications with endpoints in the *his*G–*his*D spacer might be less subject to such polarity effects. Alternatively, the *his*G–*his*D intercistronic spacer might be quite large. However, such a large region has not been revealed genetically (Grabnar *et al.*, 1964).

This work was supported by grants from the United States Public Health Service (GM-18663) and from the National Science Foundation (PCM76-15048). One author (R. P. A.) was supported by a National Science Foundation predoctoral fellowship.

REFERENCES

Ahmed, A. (1975). *Mol. Gen. Genet.* **136**, 243–253.

Ames, B. N., Hartman, P. E. & Jacob, F. (1963). *J. Mol. Biol.* **7**, 23–42.

Anderson, R. P. & Roth, J. R. (1976). *Abstr. Annu. Meeting, Amer. Soc. Microbiol.* p. 107. American Society for Microbiology, Washington, D.C.

Anderson, R. P. & Roth, J. R. (1977*a*). *Annu. Rev. Microbiol.* **31**, 473–505.

Anderson, R. P. & Roth, J. R. (1977*b*). *Abstr. Annu. Meeting, Amer. Soc. Microbiol.* p. 139. American Society for Microbiology, Washington, D.C.

Anderson, R. P., Miller, C. G. & Roth, J. R. (1976). *J. Mol. Biol.* **105**, 201–218.

Atkins, J. F. & Loper, J. C. (1970). *Proc. Nat. Acad. Sci., U.S.A.* **65**, 925–932.
Bachmann, B. J., Low, K. B. & Taylor, A. L. (1976). *Bacteriol. Rev.* **40**, 116–167.
Basu, S. K. & Margolin, P. (1972). *Genetics*, **71**, Suppl., 3, 3.
Basu, S. K. & Margolin, P. (1973). *Abstr. Annu. Meeting, Amer. Soc. Microbiol.* p. 71, American Society for Microbiology, Washington, D.C.
Beeftinck, F., Cunin, R. & Glansdorff, N. (1974). *Mol. Gen. Genet.* **132**, 241–253.
Bertani, G. & Bertani, L. E. (1974). *Proc. Nat. Acad. Sci., U.S.A.* **71**, 315–319.
Campbell, A. (1963). *Virology*, **20**, 344–356.
Campbell, A. (1965). *Virology*, **27**, 329–339.
Chattoraj, D. K. & Inman, R. B. (1974). *Proc. Nat. Acad. Sci., U.S.A.* **71**, 311–314.
Cunin, R., Elseviers, D. & Glansdorff, N. (1970). *Mol. Gen. Genet.* **108**, 154–157.
Elseviers, D., Cunin, R. & Glansdorff, N. (1969). *FEBS Letters*, **3**, 18–20.
Elseviers, D., Cunin, R., Glansdorff, N., Baumberg, S. & Ashcroft, E. (1972). *Mol. Gen. Genet.* **117**, 349–366.
Franklin, N. C. (1971). In *The Bacteriophage Lambda* (Hershey, A. D., ed.), pp. 175–194, Cold Spring Harbor Laboratory, Cold Spring Harbor.
Franklin, N. & Luria, S. (1961). *Virology*, **15**, 299–311.
Freedman, R. & Brenner, S. (1972). *J. Mol. Biol.* **69**, 409–419.
Glansdorff, N. & Sand, G. (1968). *Genetics*, **60**, 257–268.
Grabnar, M., Stahl, R. C., Hartman, Z. & Hartman, P. E. (1964). *Brookhaven Symp. Biol.* **17**, 15–52.
Hegeman, G. D. & Rosenberg, S. L. (1970). *Annu. Rev. Microbiol.* **24**, 429–462.
Hershey, A. D. (1970). *Yearb. Carnegie Inst.* **69**, 717–722.
Hill, C. W. & Echols, H. (1966). *J. Mol. Biol.* **19**, 38–51.
Hill, C. W., Schiffer, D. & Berg, P. (1969). *J. Bacteriol.* **99**, 274–278.
Hill, C. W., Grafstrom, R. H., Harnish, B. W. & Hillman, B. S. (1977). *J. Mol. Biol.* **116**, 407–428.
Hilton, J., Kearney, P. & Ames, B. N. (1965). *Arch. Biochem. Biophys.* **112**, 544–547.
Ino, I., Hartman, P. E., Hartman, Z. & Yourno, J. (1975). *J. Bacteriol.* **123**, 1254–1264.
Jackson, E. N. & Yanofsky, C. (1973). *J. Bacteriol.* **116**, 33–40.
Jacob, F. & Monod, J. (1961). *Cold Spring Harbor Symp. Quant. Biol.* **26**, 193–211.
Kleckner, N., Chan, R. K., Tye, B. K. & Botstein, D. (1975). *J. Mol. Biol.* **97**, 561–575.
Levinthal, M. & Yeh, J. (1972). *J. Bacteriol.* **109**, 993–1000.
Margolin, P. & Bauerle, R. H. (1966). *Cold Spring Harbor Symp. Quant. Biol.* **31**, 311–320.
McEntee, K. (1976). *Virology*, **70**, 221–222.
Miller, C. G. & Roth, J. R. (1971). *J. Mol. Biol.* **59**, 63–75.
Miller, J. H., Reznikoff, W. S., Silverstone, A. E., Ippen, K., Signer, E. R. & Beckwith, J. R. (1970). *J. Bacteriol.* **104**, 1273–1279.
Morse, M. L. (1967). *Genetics*, **56**, 331–340.
Ohno, S. (1970). *Evolution by Gene Duplication*, Springer Verlag, New York.
Ohtsubo, E. (1970). *Genetics*, **64**, 189–197.
Roth, J. R. (1970). *Methods Enzymol.* **17**, 3–35.
Sanderson, K. & Hartman, P. E. (1978). *Bacteriol. Rev.* **42**, in the press.
Schmieger, H. (1971). *Mol. Gen. Genet.* **110**, 378–381.
Scott, J. F., Artz, S. W. & Roth, J. R. (1975). *Proc. Nat. Acad. Sci., U.S.A.* **72**, 5021–5025.
St. Pierre, M. L. (1968). *J. Mol. Biol.* **38**, 71–82.
Straus, D. S. & Hoffmann, G. R. (1975). *Genetics*, **80**, 227–237.
Straus, D. S. & Straus, L. D. (1976). *J. Mol. Biol.* **103**, 143–154.
Vogel, H. & Bonner, D. (1956). *J. Biol. Chem.* **218**, 97–106.
Witkin, E. W. (1976). *Bacteriol. Rev.* **40**, 869–907.
Yudkin, M. D. (1977). *J. Bacteriol.* **130**, 57–61.