JOURNAL OF BACTERIOLOGY, Dec. 1978, p. 1094-1108
0021-9193/78/0136-1094$02.00/0

Vol. 136, No. 3
Printed in U.S.A.

Duplications of Histidine Transport Genes in *Salmonella typhimurium* and Their Use for the Selection of Deletion Mutants

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Received for publication 5 July 1978

We demonstrate that tandem duplications of the histidine transport operon can be selected by requesting elevated levels of transport activity to be present. Several strains were constructed which contain duplications heterozygotic for either *hisJ*, *hisQ*, or *hisP*. The size of one duplication which was analyzed in detail is about 16 genes, with one end close to the promoter site (*dhuA*) of the histidine transport operon and, therefore, enclosing about 12 more genes counterclockwise to this operon. Duplication-carrying strains could be utilized for the selection of deletion mutations by requiring both copies of the operon to be rendered defective simultaneously and, therefore, unable to transport into the cell an inhibitory histidine analog, α-hydrazino imidazole propionic acid. Over 60% (probably as high as 100%) of the α-hydrazino imidazole propionic acid-resistant strains arising in the selection are deletion mutants. The principle of our selection method is generally applicable and will be useful in the accumulation of deletions for mapping and fusing of genes and other purposes.

In our work on the mechanism of histidine transport in *Salmonella typhimurium*, we have recognized the existence of several proteins which are components of the high-affinity histidine transport system. Two of these proteins have been identified: the periplasmic histidine-binding protein J (product of the *hisJ* gene) and the P protein (product of the *hisP* gene). The product of the *hisQ* gene has not yet been identified. The *hisJ*, *hisP*, and *hisQ* genes, together with a regulatory site, *dhuA* (D-histidine utilization; see below for a description of the properties of *dhuA*), apparently form an operon (Fig. 1; [6]). The phenotypes of *hisP* and *hisQ* mutants are identical (5). In fact, until recently the *hisQ* and *hisP* genes were not identified as separate entities, but together were thought to constitute a single gene (previously defined as *hisP*). However, we found that this gene codes for at least two separate products, a Q protein and a P protein, and, therefore, it has been divided into the *hisQ* and *hisP* genes. The J protein has been purified and characterized (15), whereas the P protein has been identified only recently (5).

As part of an effort to isolate strains producing elevated levels of histidine transport components, we obtained strains carrying chromosomal duplications of the histidine transport operon. Chromosomal duplications have been isolated and characterized in *S. typhimurium*, *Escherichia coli*, and other organisms and have been shown to occur at very high frequency and to be of a variety of sizes, some extremely large (reviewed in reference 9). This paper describes the isolation and characterization of our duplication-carrying strains, which will be useful for the study of the regulation and function of the histidine transport operon. Moreover, the availability of these duplications has allowed us to develop a selection procedure which yields deletion mutations at very high frequency. The method should be applicable to any case where a positive selection is available for a defective gene and the defect is recessive.

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MATERIALS AND METHODS

Media and growth conditions. Medium E (23) containing 0.4% glucose was used as minimal medium. When required, this medium was supplemented with 10^{-4} M L-histidine. Difco nutrient broth (0.8%) with 0.5% NaCl was used as complex medium. Solid media contained 1.5% Difco agar (minimal medium) or 1.0% Iono agar (complex medium). Liquid cultures were aerated on a gyratory shaker. Tetracycline-hydrochloride was used at 25 μg/ml in solid complex medium. The inhibitory histidine analog α-hydrazino imidazole propionic acid (HIPA) was synthesized as described (20) and used as specified. L-Histidine was replaced on solid media by carnosine (3×10^{-5} M) or D-histidine

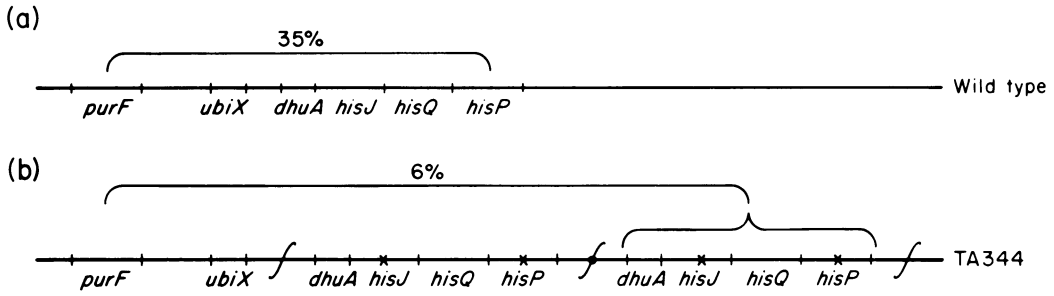

FIG. 1. *Genetic map of the histidine transport operon region. (a) Wild type. (b) Tandem duplication covering the histidine transport operon (TA344). The percent values are the linkage values obtained with P22 phage. The squiggle with a dot in the center is the join point of the duplication (i.e., the site where the duplicated material is joined to the original copy). The other two squiggles indicate the end points of the duplicated material. The join point has been placed between hisP and the duplicated dhuA at an arbitrary distance from either; its exact location is not known. The drawing is not to scale for the length of material intervening between the end of the first hisP gene and the join point and between this latter and the second copy of the dhuA gene. All dhuA sites carry the promoter-up mutation dhuA1; this is omitted from the figure for simplicity. The point mutation in hisJ is hisJ5625; the point mutation in hisP is hisP5700. Site ubiX is a gene apparently involved in ubiquinone biosynthesis (6).*

(amounts as specified) as a source of histidine. Phage P22 HT *int*-201 (6, 19) was used for all transduction experiments. All genetic techniques have been described (17).

Bacterial strains. All strains are derived from *S. typhimurium* strain LT2. Table 1 lists all strains used in this work. Mutations in *dhuA*, the promoter site for the histidine transport operon, allow growth of a histidine auxotroph on D-histidine as an L-histidine source (4) by elevating the level of D-histidine transport, which is limiting in D-histidine utilization (4, 13).

Strain TA344 was isolated from the poor D-histidine grower TA342 by selecting for fast-growing papillae around a disk of D-histidine (1 μmol) on minimal plates. The construction of other duplication-carrying strains is described in the text.

The selection for HIPA-resistant derivatives of TA3140 and TA344 was performed as follows. Nutrient broth cultures derived from individual single colonies were plated by the soft agar overlayer method (17) on minimal carnosine plates, with the last-minute addition of 0.15 ml of 100 mg of HIPA per ml. After incubation at 37°C for 2 days, one colony from each of the plates was picked and purified twice on nutrient broth plates. In cases where resistant colonies of different morphology (such as apparent excretors) were obvious, one colony of each type was picked and purified. Each colony was tested by the radial streak method (17) for its resistance to HIPA and its ability to utilize D-histidine as a histidine source.

Derivatives of TA3276 and TA3277 carrying *recA1* were built by transducing them to tetracycline resistance with phage prepared on TT521 (*srl-2*::Tn*10 recA1 rpsL*; kindly supplied by J. R. Roth) and screening the transductants for UV light sensitivity (i.e., the *recA* phenotype); the *srl* and *recA* genes are closely linked to each other.

All strains were analyzed by transductional mapping as described (6).

Analytical methods. Cells were osmotically shocked, and the level of histidine-binding activity in the shock fluid was analyzed as described (14). Electrophoresis on sodium dodecyl sulfate–polyacrylamide slab gels was performed on either the shock fluid or whole cells as described (3).

RESULTS

Identification of a mutation causing elevated levels of J protein. We have shown previously that the J and the P proteins interact directly with each other as part of their transport function (8). The J protein has been shown (14) to contain two separate sites essential for transport: the histidine-binding site and the site responsible for interacting with the P protein. A transport-defective strain, TA308, has been characterized (14) that carries a *hisJ* mutation, *hisJ5625*, which causes a defect in the interaction site of the histidine-binding protein J (J* designates this mutated protein). From TA308 we isolated a derivative with improved histidine transport, TA342. TA342 was shown (8) to carry a *hisP* mutation, *hisP5700*, which suppresses mutation *hisJ5625* by compensating for the J* defect through a mutation in the P protein. A convenient property of the J* protein is that it exhibits an altered mobility in sodium dodecyl sulfate–polyacrylamide gel electrophoresis (14), and thus its presence can be easily ascertained. Mutation *hisP5700* by itself causes only a partial defect in *hisP* function. Suppression of the transport defect is not complete in TA342, which, therefore, transports histidine poorly. As a consequence, growth of the doubly mutated strain TA342 on D-histidine as a source of L-histidine is also poor. In fact, a convenient assay for the proper functioning of the high-affinity histidine transport system is given by the ability to grow

TABLE 1. *Strains used in this study*

Strain no.	*his*Δ allele no.	Genotype[a]	Parent/remarks[b]
Δ			
TA271		*hisF645 dhuA1*	(4)
TA308		*hisF645 dhuA1 hisJ5625*	(14)
TA342		*hisF645 dhuA1 hisJ5625 hisP5700*	TA308 (8)
TA344		*hisF645 dhuA1 hisJ5625 hisP5700:dhuA1*(?) *hisJ5625 hisP5700*	TA342 (this paper)
TA3140		*hisF645 dhuA1 hisP5700:dhuA1*(?) *hisJ5625 hisP5700*	TA344 (this paper)
TA1008		*hisF645 dhuA1 hisP5503* (ΔP[c])	(4)
TA1741		*hisF645 dhuA1 his-5575* (ΔQP[c])	(6)
TA3078		*hisF645 his-8907* (ΔUAJ[c])	(16); tetracycline-resistant excision derivative from a Tn*10*-carrying strain
TA3067		*hisF645 dhuA1 hisQ6757*::Tn*10*	(6)
TA3367		*hisF645 dhuA1 hisJ5625 hisQ6757*::Tn*10:dhuA1 hisJ5625 hisP5700*	This paper
TA3368		*hisF645 dhuA1 hisQ6757*::Tn*10:dhuA1 hisJ5625 hisP5700*	This paper
Carrying new deletion[d]			
TA3141	*6800*	(ΔAJ) *hisJ5625 hisP5700*	TA344
TA3246	*8951*	(ΔAJQP)	TA3140
TA3247	*8952*	(ΔAJ) *hisJ5625 hisP5700*	TA3140
TA3248	*8953*	(ΔAJQP)	TA344
TA3250	*8955*	(ΔAJQP)	TA3140
TA3251	*8956*	(ΔQP) *hisJ5625 hisP5700*	TA3140
TA3252	*8957*	(ΔAJQP)	TA3140
TA3253	*8958*	(ΔUAJQP) *hisP5700*(?)	TA3140
TA3254	*8959*	(ΔQ) *hisP5700*	TA3140
TA3255	*8960*	(ΔAJQP)	TA3140
TA3256	*8961*	(ΔAJQP)	TA3140
TA3257	*8962*	(ΔAJQP)	TA3140
TA3258	*8963*	(ΔP) *hisP5700*(?)	TA3140
TA3259	*8964*	(ΔUAJQP fluoroacetate[r])	TA3140
TA3260	*8965*	(ΔAJQP)	TA3140
TA3261	*8966*	(ΔQP) *hisP5700*	TA3140
TA3262	*8967*	(ΔAJQP)	TA3140
TA3263	*8968*	(ΔAJ)	TA3140
TA3264	*8969*	(ΔP) *hisP5700*	TA3140
TA3265	*8970*	(ΔP?) *hisJ5625 hisP5700*(?)	TA344
TA3266	*8971*	(ΔP?) *hisJ5625 hisP5700*(?)	TA344
TA3267	*8972*	(ΔP?) *hisJ5625 hisP5700*	TA344
TA3268	*8973*	(ΔQ?) *hisJ5625 hisP5700*	TA344
TA3269	*8974*	(ΔJ?) *hisJ5625 hisP5700*	TA3140
TA3270	*8975*	(ΔQ?) *hisP5700*	TA3140
TA3271	*8976*	(ΔQ?) *hisP5700*	TA3140
TA3272	*8977*	(ΔP?) *hisP5700*	TA3140
TA3273	*8978*	(ΔQ?) *hisJ5625 hisP5700*	TA344
TA3274	*8979*	(ΔQ?) *hisP5700*	TA3140
TA3275	*8980*	(ΔP?) *hisP5700*	TA3140
TA3276	*8981*	See text	TA3140
TA3277	*8982*	See text	TA3140

[a] Mutation *hisF645* is a deletion in the histidine biosynthetic operon. Duplications are described by repeating after a colon the names of the genes known to have been duplicated.

[b] Numbers in parentheses indicate references when the strain was first described.

[c] Genes *dhuA*, *hisJ*, *hisQ*, *hisP*, and *ubiX* are abbreviated as A, J, Q, P, and U, respectively, in the description of markers covered by the deletion.

[d] All these strains carry *hisF645* and a promoter-up mutation, *dhuA1*, in the histidine transport operon unless *dhuA* has been deleted.

on D-histidine as a source of L-histidine, because the limiting step in this process is the rate of uptake of D-histidine through this system. An elevation of transport would result in improved D-histidine growth and could be achieved by elevating the level of both altered J and P proteins. Therefore, to elevate the level of synthesis of the transport components J and P, we selected for mutants of TA342 which would be able to grow faster on D-histidine (see above). One of the clones, TA344, has been characterized further and will be shown below to be a duplication mutant. Other clones with apparently similar properties were obtained on several occasions but were not characterized further.

The rate of growth of TA344 on L- and D-histidine, as compared with its parent, TA342, and to its grandparent, TA308, is shown in Table 2. It can be seen that TA344 grows much better on D-histidine than does TA342 and that TA308, as expected, does not grow at all. Growth of TA344, however, is not as good as that of TA271, which has wild-type J and P proteins. Growth on L-histidine, the transport of which occurs through multiple systems (2), is unaffected in all of these strains.

The level of the J* protein was assessed both by direct assay of the J protein in the crude shock fluid and by inspection of sodium dodecyl sulfate–polyacrylamide gels of whole cells (data not shown) and crude shock fluids (Fig. 2). A band of increased intensity was clearly visible in TA344 in the area of the altered J* protein (which has a slower mobility in these gels than the wild-type J protein), indicating that the mutant protein was still present and in increased amounts. The two strains analyzed in wells 5 and 6 are other D-histidine fast-growing clones which have similar properties. They were not analyzed further. Assay in crude shock fluids of histidine-binding activity (more than 90% of which is due to the J protein activity [4]) yielded a value two- to fourfold higher than the value

TABLE 2. *Growth rates*[a]

Strain	Doubling time (h)		
	D-histidine (5×10^{-5} M)	D-histidine (1.5×10^{-4} M)	L-histidine (10^{-4} M)
TA271	0.9	1.0	1.2
TA308	No growth (>24 h)	>10	1.1
TA342	5.5	1.9	1.0
TA344	1.5	1.1	1.2

[a] Cultures were grown to saturation in minimal medium containing L-histidine (10^{-4} M) and centrifuged, and the pellets were resuspended in minimal medium without histidine. Fiftyfold dilutions were made into media containing D- or L-histidine as indicated.

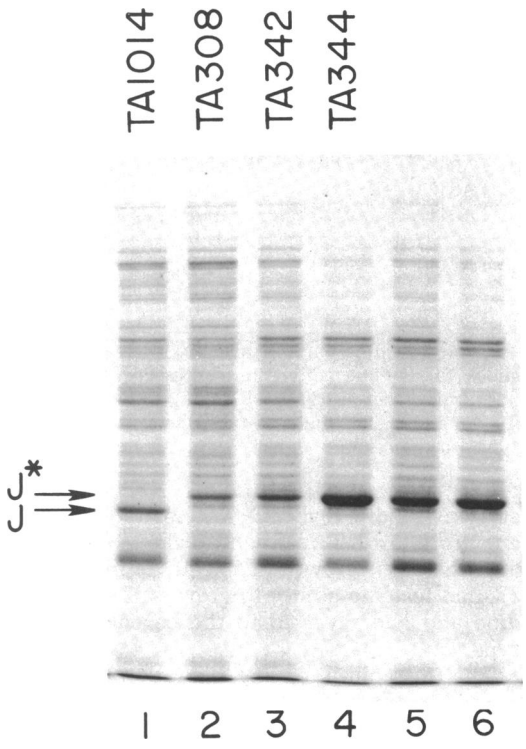

FIG. 2. *Sodium dodecyl sulfate-polyacrylamide gel electrophoresis of shock fluids from duplication-carrying and parental strains. TA1014 (dhuA1), which is a His$^+$ derivative of TA271, is a control strain, containing the same amount of J as TA308. TA308, however, carries mutation hisJ5625 and, therefore, produces altered J protein (J*). TA308 is the parent of TA342, which in turn is the parent of TA344 and of the two strains in wells 5 and 6. The latter three strains overproduce the J* protein and are candidates for carrying duplication mutations. The acrylamide concentration was 10%. The anode is at the bottom. The genotypes of all strains appear in Table 1.*

obtained with the parental and related strains which do not carry duplications (Table 3). Later we discuss the possible significance of this larger than twofold elevation (see below).

Thus, a mutation is present in the TA344 strain which causes an increased level of J protein and of D-histidine transport. Its genetic properties described below indicate that this mutation is a duplication.

Instability of TA344. Because one of the characteristics of duplications is their instability (9), this property was studied in TA344 by allowing the cells to replicate numerous times in a medium not selective for maintenance of the duplication. After overnight growth in nutrient broth (corresponding to approximately 10 gen-

TABLE 3. *Histidine-binding activity*[a]

Strain	Type of J protein	Histidine-binding activity		
		Avg		%
TA271	J	14.2 11.5	12.8	100
TA308	J*	13.1		102
TA342	J*	10.9		85
TA344	J*/J*	45.1 47.9	46.5	360
TA3140	J*/J	25.9 30.0	28.0	220
TA1008	J	11.03		86

[a] The histidine-binding activity was assayed on shock fluids. The activity is expressed as units per absorbance unit at 650 μm. The J* protein present in TA308 and TA342 binds histidine as well as the wild-type J protein (14).

erations), individual cells were plated on nutrient broth, grown overnight, picked, and tested for D-histidine growth (Table 4); approximately 5% had lost the ability to grow well on D-histidine and had reverted to the parental phenotype.

The instability of the mutation could be greatly increased by treating the cells with the frameshift mutagen ICR-191. Approximately 27% of the TA344 cells lost the ability to grow well on D-histidine after ICR-191 mutagenesis and growth in a nonselective medium. Eleven ICR-191-induced derivatives were obtained in a separate experiment (by penicillin selection on D-histidine) as having lost the ability to grow on D-histidine; when analyzed by electrophoresis, they all had lost the J* elevation.

The fact that TA344 readily loses its D-histidine growth properties and reverts to its parental phenotype is consistent with our hypothesis that it contains an unstable duplication which is responsible for the J* elevation.

Mapping properties of TA344. The histidine transport operon, located at unit 49 on the *S. typhimurium* map (6, 18), is 35% linked by phage P22 cotransduction to *purF*. However, the linkage to *purF* of the mutation responsible for good D-histidine growth in TA344 was 6% (as obtained by transducing TA1637 [*purF145* Δ*hisF645*] to Pur$^+$ with phage prepared on TA344 on an L-histidine plate and screening the Pur$^+$ transductants for growth on D-histidine); in two experiments the Pur$^+$ D-histidine growers were 2/30 and 1/20, respectively. This result indicates that in TA344 the chromosomal location of the event resulting in improved D-histidine growth is farther away from *purF* than the transport operon. Although the reduced cotransducibility could be interpreted in a variety of ways, one consistent explanation is that the size of the entire duplication present in TA344 is such that the most distant point which needs to be transduced to give D-histidine growth has a linkage to *purF* of 6% (Fig. 1). The decreased linkage thus can be an indication of the size of the fragment which has been duplicated (see below).

TABLE 4. *Instability of TA344 and TA3140*

Strain	Genotype[a]	Growth ability of nutrient broth clones[b]			
		D-histidine[c]		L-histidine	% of poor D-histidine utilizers
		good	poor		
TA342	J*P*	0 0	51 47	51 47	100[d] 100[d]
TA344	J*P*/J*P*	48 45	3 2	51 47	6 4
TA344 + ICR-191[e]		37	14	51	27
TA3140	J*P*/JP*	282	5	287	2
TA3140 + ICR-191		55	26	81	32
Recombinant 1[f]	J*P*/JP*	278	3	281	1
Recombinant 1[f] + ICR-191		39	26	65	40

[a] The relevant genotype is schematically represented as: J* (*hisJ5625*), J (wild-type *hisJ*), P* (*hisP5700*), P (wild-type *hisP*); letters after the slash refer to the duplication of those genes under examination: of course, the duplication also covers other genes (*dhuA*, *hisQ*, and other unknown ones).

[b] Colonies arising from individual cells from an overnight nutrient broth culture were picked from nutrient broth plates and tested first for D-histidine and then for L-histidine growth. Results are presented for two separate experiments, except for the ICR-191-treated culture.

[c] TA342 has a poor, but clearly distinguishable, ability to grow on D-histidine. Those clones of TA344 which have lost the ability to grow well on D-histidine behave similarly to TA342 with respect to D-histidine growth.

[d] TA342, as expected, is a poor grower, and no change occurs during dilution and growth overnight.

[e] A culture of TA344 was treated with ICR-191 as described (1). The mutagenized culture was diluted in nutrient broth, fully grown, and then tested as described in footnote *b*.

[f] Sibling obtained from same transductional cross from which TA3140 was obtained (see text) and presumably identical to it.

Isolation of *hisP* mutants in TA344. Mutations in *hisP* or *hisQ* (which have the same phenotype) are recessive to the wild-type allele (7). This is easily accounted for by the transport function which the products of these genes perform. The wild-type alleles allow active transport of substrates, even in the presence of a nonfunctional second copy of the gene. Therefore, in the case of an inhibitory histidine analog transported by this system, transport will occur, and analog sensitivity will be expressed in a

transport$^+$/transport$^-$ heterozygote. As a consequence, in a strain carrying a duplication of the *hisP* and *hisQ* genes, it is not possible to obtain resistance to the inhibitory histidine analog HIPA as a result of a single point mutation (7). In this case, resistance could occur only through a double event or in a deletion mutant (see below), damaging both copies of the genes simultaneously.

In agreement with our hypothesis that TA344 contains two copies of the *hisP* gene (both presumably carrying the *hisP5700* mutation, which only partly eliminates transport of HIPA) is the fact that the frequency of appearance of HIPA-resistant colonies in a zone of HIPA inhibition is low as compared with a wild type. The number of HIPA-resistant clones appearing for the wild type in a zone of HIPA inhibition (see below) varies depending on the individual culture used (from a few to approximately 50). However, in repeated selections attempted with TA344, only an occasional resistant clone was visible. One such clone (TA3141, carrying Δ*his-6800*; see Fig. 8) was analyzed genetically and shown to be a deletion extending out of the operon on the left and deleting up to part of region VIII in *hisJ*; it, of course, no longer carries the duplication. The possibility that duplication-carrying strains might be useful in the selection of deletion mutants was therefore explored (see below).

Construction of TA3140, a duplication strain containing two different alleles of the *hisJ* gene. All the characteristics found for TA344 (i.e., elevated levels of a gene product, instability, difficulty in isolating HIPA-resistant mutants, and mapping properties) indicated that it contained a duplication of the histidine transport operon. As a final confirmation that TA344 had two copies of each transport gene, we felt that we should be able to construct from it a strain in which the two gene copies produced distinguishable products. This could be accomplished in the case of the *hisJ* gene, the product of which we can analyze easily by electrophoresis. Thus, we replaced one of the two *hisJ5625* genes with a wild-type copy by transducing a *hisP* deletion mutant to growth on D-histidine with a phage prepared on TA344. A model show-

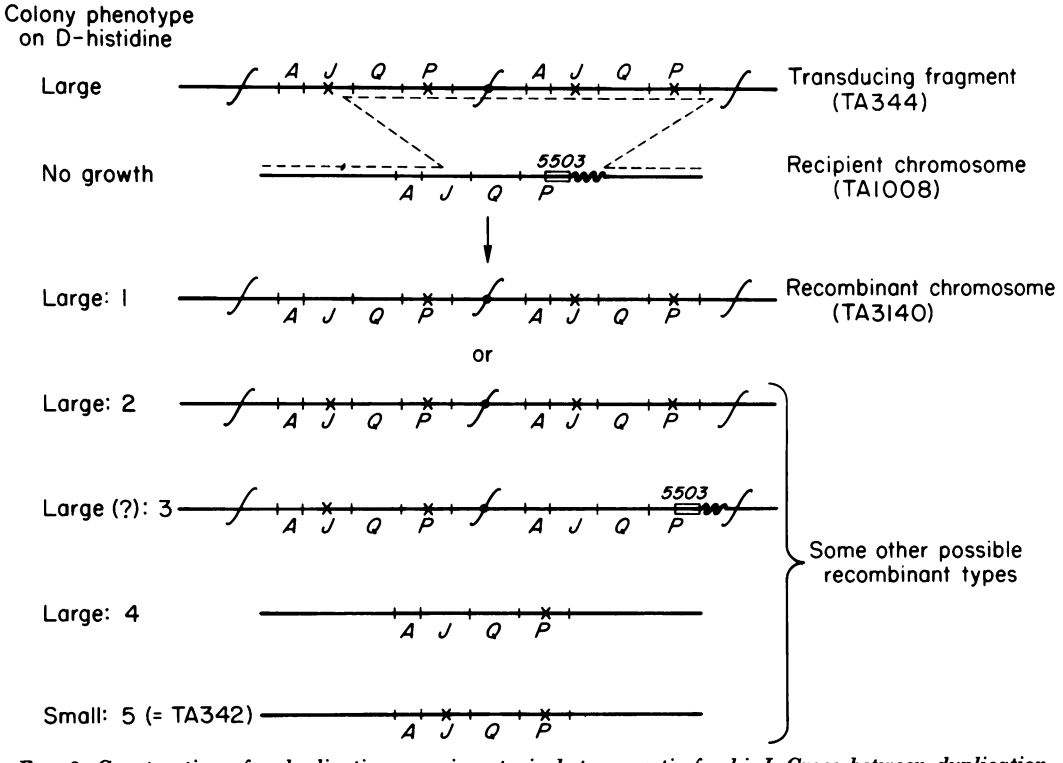

FIG. 3. *Construction of a duplication-carrying strain heterozygotic for hisJ. Cross between duplication-carrying strain TA344 and hisP deletion-carrying strain TA1008. Only some of the possible recombinants arising from this cross are pictured. The size of the colonies on a D-histidine plate is listed for each recombinant. Recombinant on line 1 (TA3140) was the one selected for further study. Conventions used are: dhuA, A; hisJ, J; hisQ, Q; hisP, P. The dashed lines represent recombination events. Other conventions are described in the legend to Fig. 1.*

ing how the appropriate recombinant could arise appears in Fig. 3. Strain TA1008, which contains a deletion (Δ*hisP5503*) covering *hisP5700* was the recipient in the transduction, which yielded two types of transductants: small colonies and large colonies. The small colonies probably acquired a single copy of the operon and contain both the *hisJ5625* mutation and the compensating *hisP5700* mutation (thus being identical to TA342 and growing poorly on D-histidine; last line in Fig. 3). The large colonies could be of several types: (i) they could be of the parental TA344 genotype (Fig. 3; i.e., identical to the transducing fragment); (ii) they could have acquired a single copy of the operon and contain, by recombination, a wild-type *hisJ* gene and *hisP5700* (line 4 in Fig. 3); or (iii) they could still contain a duplication composed of a variety of *hisJ* and *hisP* gene combinations (lines 1 to 3 in Fig. 3 are some examples). The possible recombinants still containing a duplication could be carrying the *hisJ5625* gene, besides a wild-type *hisJ* gene acquired from the recipient TA1008 parent, plus either two *hisP5700* alleles or one *hisP5700* allele and one Δ*hisP5503* allele recovered from TA1008 (the combination of the wild-type *hisJ* gene with the partially defective *hisP5700* allele produces a strain described by line 4 in Fig. 3, which grows better than TA344 on D-histidine). No true wild type could arise from this cross. To analyze further the large-colony-size recombinants obtained, six were purified and analyzed by electrophoresis (Fig. 4, wells 2 through 7). Two of the six recombinants (wells 4 and 7) contained both altered (J*) and wild-type J protein bands. The other four looked identical to TA344 in having an elevated amount of the J* protein band and no wild-type J, and were discarded. The two recombinants exhibiting both J protein bands were analyzed further. Only one of these, TA3140 (well 7), was eventually saved.

The instability of both these recombinants was assessed as described above, and the results are shown in Table 4 (last four lines): about 1 to 2% (5/287 in one case and 3/281 in the other) of the cells had lost the ability to grow well on D-histidine. When cultures of both transductants were treated with mutagen ICR-191, the loss of good D-histidine growth ability was 32% (26/81) and 40% (26/65), respectively. Thus, both the spontaneous and the mutagen-induced instability were similar to the values obtained for the parental duplication TA344.

The level of histidine-binding activity in TA3140 was assayed (Table 3) and found to be about twofold higher than that of strains not carrying duplications.

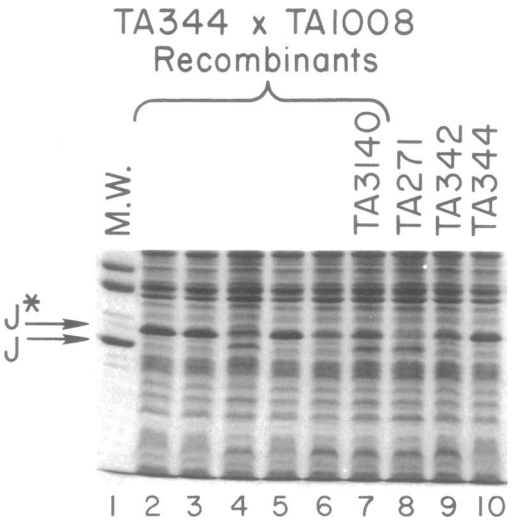

FIG. 4. *Sodium dodecyl sulfate-polyacrylamide gel electrophoresis of duplication-carrying strains heterozygotic for hisJ. The electrophoresis was performed on whole cells, on 10% acrylamide; only the bottom half of the gel is shown. The front (anode) is at the bottom. Well 1 contains molecular weight (MW) standards, of which the following three are visible (from top to bottom: horse liver alcohol dehydrogenase, 41,000; glyceraldehyde phosphate dehydrogenase, 36,000; and wild-type J protein, 25,000). Wells 2 to 7 contain six different recombinants (which gave large-size colonies on D-histidine) arising from the cross between TA344 and TA1008 (see Fig. 3). The strain in well 7 (TA3140) was the one chosen for further work. The strain in well 5 seems identical to TA3140 and was not studied further. The genotypes of all strains mentioned are in Table 1.*

All of the above data indicate that TA3140 (and recombinant in well 4, Fig. 2) carries a duplication which contains both a wild-type and a mutant allele of the *hisJ* gene. However, it does not give any information as to the nature of the two copies of the *hisP* gene. We attempted to solve this problem by examining TA3140 for the presence of deletion *his-5530*. If Δ*hisP5503* is part of the genotype of TA3140, it should be possible to recover it either among the segregants derived from loss of the duplication or by genetic crosses. No complete loss of D-histidine growth (which is the characteristic of a strain containing a *hisP* deletion) was encountered among the spontaneous or mutagen-induced segregants; 281 individual colonies were screened, out of which 31 segregants were obtained, and they were all of the TA342 type. Attempts at recovering the *hisP* deletion (Δ*hisP5503*) by transducing it (if it were present) into a PurF$^-$ strain failed: <2% (0/60) of Pur$^+$ transductants were HisP$^-$. It is possible that the

deletion is located in the copy of the operon which is distal from *purF* and very far from the join point of the duplication; if this were the case, it might be poorly cotransducible with *purF* (but see below). This alternative was not investigated further because of the lack of appropriate markers counterclockwise from the histidine transport operon. TA3140 was tentatively assumed to contain no *his-5503* deletion, although it is possible that Δ*hisP5503* is present, but not easily recoverable by our presently available methods (see Fig. 7 and relevant discussion).

Thus, on the basis of the above results, TA3140 probably also carries two identical copies of the mutant *hisP* gene (*hisP5700*), because it does not seem to carry the *his-5503* deletion, and no wild-type *hisP* gene could have been present because *hisP5700* maps in region XII, which is covered by deletion *his-5503*.

Construction of duplication strains containing two different alleles of the *hisQ* and *hisP* genes. A second approach, which is based on the utilization of the tetracycline resistance transposon Tn*10* for genetic manipulations (12), was used to construct a heterozygotic duplication. Strains carrying a Tn*10* transposon inserted in one of their genes have two important characteristics: (i) the gene carrying the transposon is completely inactivated and (ii) the mutated gene can be transferred to a recipient simply by selecting for tetracycline resistance. TA344, presumed to contain the homozygous duplication, was transduced to tetracycline resistance on D-histidine plus tetracycline, with phage prepared on TA3067, which contains a Tn*10* insertion in *hisQ* (*hisQ6757*::Tn*10*). Figure 5a shows some of the possible recombinants arising from this cross. The selection requires not only that recombinants contain the tetracycline transposon located in the *hisQ* gene, but also that they be able to grow on D-histidine. As expected, the tetracycline-resistant recombinants which were purified and examined further displayed a vari-

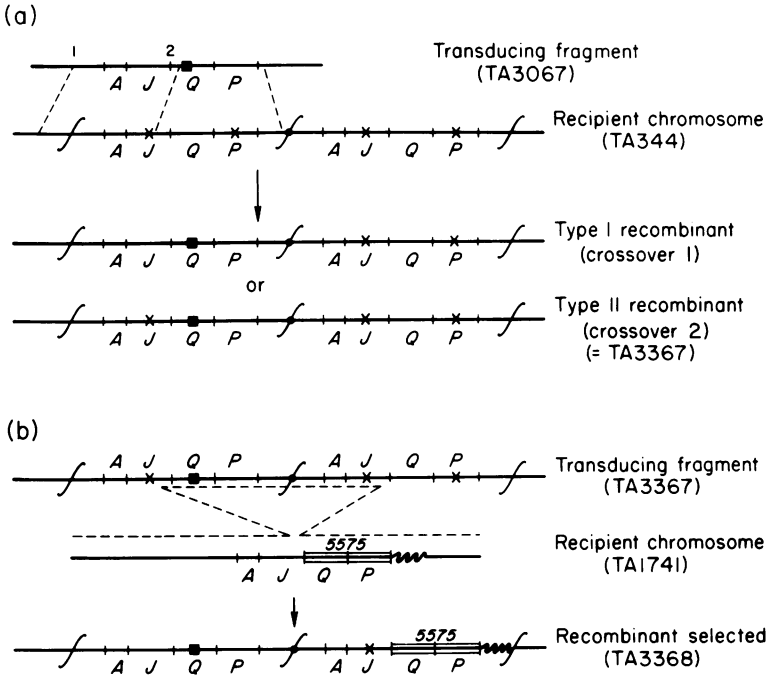

FIG. 5. *(a) Construction of duplication-carrying strains heterozygotic for hisQ and hisP. Cross between duplication-carrying strain TA344 and Tn10-carrying strain TA3067. Only two possible types of recombinants are shown; other types of duplication-carrying recombinants could arise, with different combinations of the hisJ, hisQ, and hisP genes, and they always carry the Tn10 transposon and a hisP5700 mutation (for both of which functions they were selected). (b) Cross between deletion-carrying strain TA1741 and duplication-carrying strain TA3367; only the recombinant of interest (tetracycline-resistant, D-histidine nongrower) is shown. The solid rectangle represents the Tn10 transposon (hisQ6757::Tn10); the deletion mutation in TA1741 is ΔhisQP5575; the dashed lines indicate possible sites of crossovers. All other symbols are as described in the legends to Fig. 1 and 3. The structure of TA3367 is inferred to be that of type II.*

ety of D-histidine-utilizing abilities, which ranged from very good to very poor. Gel electrophoresis (not shown) of 19 recombinants chosen among the best D-histidine utilizers indicated that they could be divided into two types, depending on the nature and level of the J protein. Type I (16 clones) had normal levels of the wild-type J protein and elevated levels of the J* protein; thus, they contain two copies of the *hisJ* gene (two different alleles) and must contain two copies of the *hisQ* and *hisP* genes: one *hisQ* gene carries the Tn*10* transposon and is therefore completely inactive and polar on the *hisP* gene present in the same operon; the duplicated segment carries a wild-type *hisQ* gene and the *hisP5700* mutation which allows some D-histidine growth. Type II (three clones) had high levels of the J* protein and no wild-type protein; these clones must have two identical copies of the mutant *hisJ* gene and must be heterozygotic for the *hisQ* and *hisP* genes (as in type I recombinants). Four type I and two type II recombinants were analyzed to determine the nature of the *hisQ* and *hisP* genes, and they yielded essentially the same results. Therefore, only the results for one of the type II recombinants (TA3367) are presented. If the *hisQ* and *hisP* structure of TA3367 were as shown in Fig. 5, it should be possible to use it as a donor of the Tn*10* transposon into a strain carrying a *hisQ* and *hisP* deletion, and among the possible recombinants there should be some which are unable to grow on D-histidine (unselected phenotypic characteristic) because both copies of *hisQ* and *hisP* genes are inactive; one is inactivated by the recipient deletion and the other by the Tn*10* transposon. The production of such a recombinant duplication from the cross can be recognized electrophoretically by the presence of the two different J proteins, with or without an additional elevation of either of them.

The cross was performed, on tetracycline medium, with TA1741 (*dhuA1* Δ*hisQP5575* Δ*hisF645*) as recipient and phage prepared on TA3367 as donor (Fig. 5b). Deletion *hisQP5575* covers the site of the Tn*10* transposon insertion. Among 15 tetracycline-resistant recombinants which were tested for D-histidine growth, three were completely unable to grow and were analyzed by gel electrophoresis (data not shown). Two of them clearly had both J* and wild-type J proteins, which indicated that they carried the duplication. However, the level of the J* protein was not elevated; this could mean that the *hisJ5625*-carrying gene is contiguous to deletion Δ*hisQP5575* (as drawn in Fig. 5b) because it is known that the presence of this deletion decreases the level of the J protein to half its normal value (G. Ferro-Luzzi Ames, unpublished data); the reason for this effect is unknown.

Thus, it was possible to obtain from TA3367 recombinants which were still heterozygotic for *hisJ*, *hisQ*, and *hisP* and which presumably were carrying both the transposon and the Δ*hisQP5575* mutation. One of these, TA3368 (Fig. 5b), was examined to verify the presence of the Δ*hisQP5575* by the use of the following two procedures.

The first procedure asked for the recovery of the Δ*hisQP5575* in a cross in which phage prepared on recombinant TA3368 was used to transduce TA271 (*dhuA1* Δ*hisF645*) to HIPA resistance (Fig. 6). Of 25 such HIPA-resistant transductants, 4 were determined to be tetracycline sensitive and were tested for the nature of the mutation causing HIPA resistance by genetic crosses. One was shown to contain a deletion with mapping properties identical to Δ*hisQP5575*. Two others behaved like deletions different from Δ*hisQP5575*. The fourth transductant behaved like a point mutant in *hisP*. Therefore, the recovery of the Δ*hisQP5575* in one of the transductants resulting from the cross shown in Fig. 6 confirmed that the recombinant duplication present in the parent TA3368 contained it as one of the two copies of the *hisP* gene. The finding of deletions, other than Δ*hisQ5575*, among the transductants of the last cross was surprising at first. However, because deletion mutants occur at high frequency in duplications (see next section), they could have

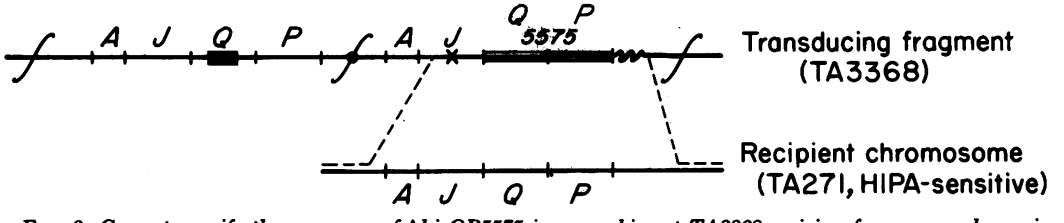

FIG. 6. *Cross to verify the presence of Δ*hisQP5575* in recombinant TA3368, arising from cross shown in Fig. 5b. See Fig. 5 for an explanation of symbols. The selection for HIPA-resistant transductants was on minimal plates, containing 2 mg of HIPA and 1 μmol of carnosine (as a source of L-histidine).*

been present in the TA3368 culture, having arisen spontaneously.

The other procedure attempting to recover *ΔhisQP5575* from TA3368 depended on the known instability of the duplication under study (about 5% loss of duplicated material; see above). A total of 150 individual clones derived from a TA3368 culture in nutrient broth was tested for tetracycline resistance. If during segregation the *hisQ/hisP* copy containing the *ΔhisQP5575* were retained and the *hisP*::Tn*10* were lost, the resulting segregants would be tetracycline sensitive but remain HisP$^-$. However, no clone turned out to be tetracycline sensitive, whereas about eight were expected if every recombinational event due to instability had occurred as shown in Fig. 7a and if the two mutated operons were in that order. This result is still in agreement with a duplication of the type suspected, if the duplication join point were closer to the operon copy carrying the *ΔhisQP5575* than to the copy carrying the *hisP*::Tn*10* (Fig. 7b); in this case recombination would occur much more frequently with the loss of the deletion than of the *hisP*::Tn*10* (see below).

In conclusion, all the available evidence indicates that both TA3367 and TA3368 are heterozygotic for *hisQ* and *hisP*.

Size of duplicated material. The inclusion in the duplication of nearby genes was tested for *purF* and *ubiX* by the method described by Hill and Combriato (10) which involves introducing the duplication in strains carrying an auxotrophic recessive marker in the gene of interest. If the duplication included this gene, then the strain would be phenotypically prototrophic, because the second wild-type copy of the gene supplies the missing function. A simple way of doing this experiment (which was suggested by J. R. Roth and R. P. Anderson) is by use of the transposable drug resistance element Tn*10*, which confers tetracycline resistance while destroying the function of the gene in which it is inserted (see review in reference 12 for uses of this element in genetic engineering). The dominance of the Tn*10* phenotype renders its presence easily selectable.

Strains TA344 and TA3140 were transduced to tetracycline resistance on rich medium either with phage prepared on TT317 (carrying a *purF*::Tn*10* marker; obtained from J. R. Roth) or with phage prepared on TA3078 (carrying a

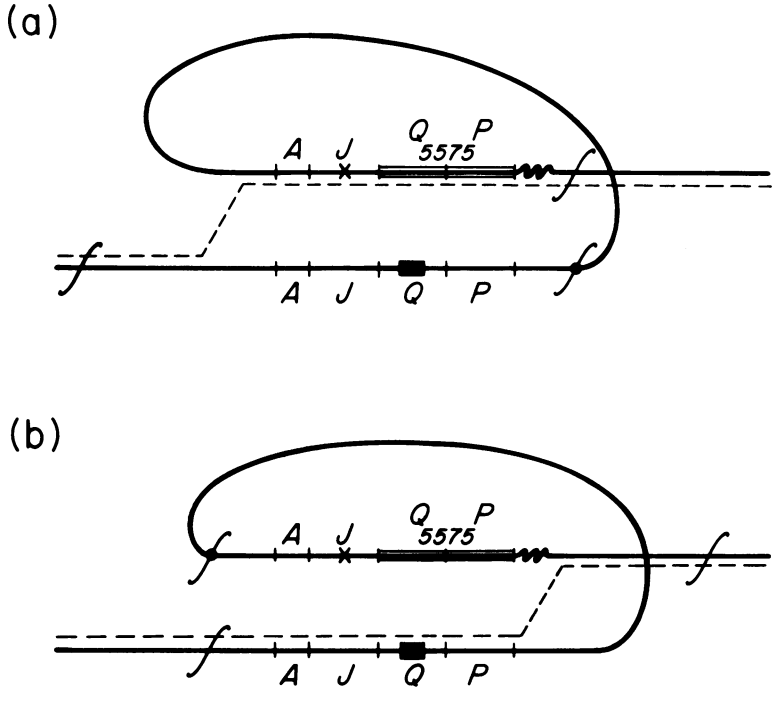

FIG. 7. *Possible recombinants arising from instability of duplication in TA3368. For symbols, see legend to Fig. 5. (a) With ample homology to the left of dhuA, chances of a crossover in that region are high. (b) If duplication join point is close to dhuA, chances of a crossover are higher in the region of homology to the right of hisP. The distance between the join point and the dhuA site has been exaggerated as being very big in (a) and very small in (b).*

deletion of *ubiX* and a part of the histidine transport operon and retaining part of the Tn*10* element from which this deletion had originated: enough to still confer tetracycline resistance [16]). Recombinants from each cross were then tested for purine auxotrophy on minimal histidine medium (if derived from TT317) or for the *ubiX* phenotype (small colony on nutrient broth [6], if derived from TA3078). All of the recombinants had inherited the parental phenotype carried by the phage. This indicates that neither *purF* nor *ubiX* is included in the duplications.

There are no close markers clockwise to the histidine transport operon. Therefore, the extent of the duplication on this side was not determined.

Selection of deletion mutants in strains carrying duplications. As discussed above, the wild-type *hisQ* and *hisP* genes are dominant. Therefore, in strains carrying two copies of functional *hisQ* and *hisP* genes, the transport function can only be eliminated by simultaneously damaging both gene copies. There are three ways in which this can occur. One is the occurrence of a deletion mutation which covers enough of the duplicated material to eliminate the function of both genes. A second one involves the loss of the duplicated material (frequency, 10^{-2}) followed by a mutational event in the remaining *hisQ* (or *hisP*) gene (frequency, $\sim 10^{-5}$; therefore, $10^{-2} \times 10^{-5} = \sim 10^{-7}$). The third requires the simultaneous occurrence of two independent mutations, one in each of the copies of the *hisQ* (or *hisP*) genes (frequency, $10^{-5} \times 10^{-5} = \sim 10^{-10}$). The first possibility is the most frequent because deletions account for about 10% of spontaneous mutations (i.e., frequency, 10^{-6}), whereas the other two possibilities should be much rarer (respectively, 10 and 10^4 times rarer). Therefore, we should have a great enrichment for deletion mutants among spontaneously arising transport-negative mutants in a duplication-carrying strain.

This was tested by selecting a total of 32 independent HIPA-resistant strains, 10 from TA344 and 22 from TA3140. Each mutant strain was derived from an individual clonal culture; in a few cases more than one strain was derived from the same parental culture because they were clearly distinguishable from each other and confirmed by mapping as being different. These 32 clones were screened for the presence of deletion mutations in the histidine transport genes. All were mapped by transduction, as donors, on D-histidine plates with numerous recipient deletion and point mutant strains appropriately chosen. They were also crossed with a DhuA$^+$ strain to determine whether the *dhuA1* allele (which they all carried initially) could be recovered. Fifty-nine percent of the total (19 of 32) emerged as clearly being deletion mutants. Three of these (*his-8959*, *his-8963*, and *his-8969*) are small deletions, as shown by their lack of recombination with two or three point mutants which map in the same region and recombine with each other. Figure 8 shows the results of these crosses. The 19 clones which are clearly deletion mutants and cover the operon to different extents are represented below the chromosomal line. Of these, five still carry a *dhuA1* allele because the deletion is contained within the operon.

Of the remaining 13 strains, the mutations in 11 of them were localized by deletion mapping to specific regions of the operon and were crossed with almost every point mutant available in that region. Of the 11, 7 (*his-8974*, *his-8970*, *his-8971*, *his-8972*, *his-8973*, *his-8975*, and *his-8976*) recombined with every point mutant in the region except one. These seven, therefore, could have arisen either as point mutants at the same site as the one with which they do not recombine, after having lost the duplication, or as small deletions covering that point mutant but none of the other ones presently available in that region. The first alternative is less likely because it involves a two-step mechanism, whereas the second one is in keeping with the finding of such a high percentage (59%) of clear-cut deletions. Therefore, we tentatively classify these mutations as deletions and represent them in Fig. 8 as horizontal bars above the chromosome, with bold vertical lines leading to the two copies of the region in which they are found to map and in which their end points are located. If these seven strains are truly deletion mutants, the percentage of deletions obtained is 81% of the total analyzed.

The remaining four clones (*his-8977*, *his-8978*, *his-8979*, and *his-8980*) carry mutations which are similar to the last seven described: they are located in specific regions, but recombine with every point mutation available in that region. Therefore, at the moment, they are tentatively classified as point mutants (which would have arisen after loss of the duplication). However, it should be stressed that they could definitely be small deletions, not covering any of the mutants used in the mapping. Until this ambiguity is resolved, these are represented in Fig. 8 by horizontal lines above the chromosome, with vertical arrows pointing to the two copies of the region in which the mutation is located; if they are point mutations, that is the region where they map; if they are deletion mutations, the end points of the deletion would be situated in that region. If the duplication was lost and then

a point mutant was created, we cannot determine which of the two operon copies is left.

Finally, the two remaining HIPA-resistant mutations (*his-8981* and *his-8982*) recombine with all deletions and point mutations tested. They behave like normal transport-negative strains: they do not grow on D-histidine as a source of L-histidine, and they are resistant to HIPA. They revert at very high frequency to growth on D-histidine (about 10^{-4}). This unusual type could be explained by assuming that a deletion has occurred which has left a partial duplication, which may be inactivating the operon genes which are genetically shown to be present. Figure 9 shows models describing this hypothetical structure. The partial duplication would be responsible for supplying intact genetic material in all crosses attempted. In Fig. 9, line I, the operon is not functional because it has no intact *hisP* gene (for example); in Fig. 9, line II, the intact *hisP* gene might be inactivated by polarity. The high reversion rate might indicate a loss of the partial duplication with consequent recovery of an intact operon; this does not seem to be the case, because introduction of a *recA* mutation (see above) did not affect the reversion rate; the product of the *recA* gene is apparently necessary for the mechanism of duplication loss

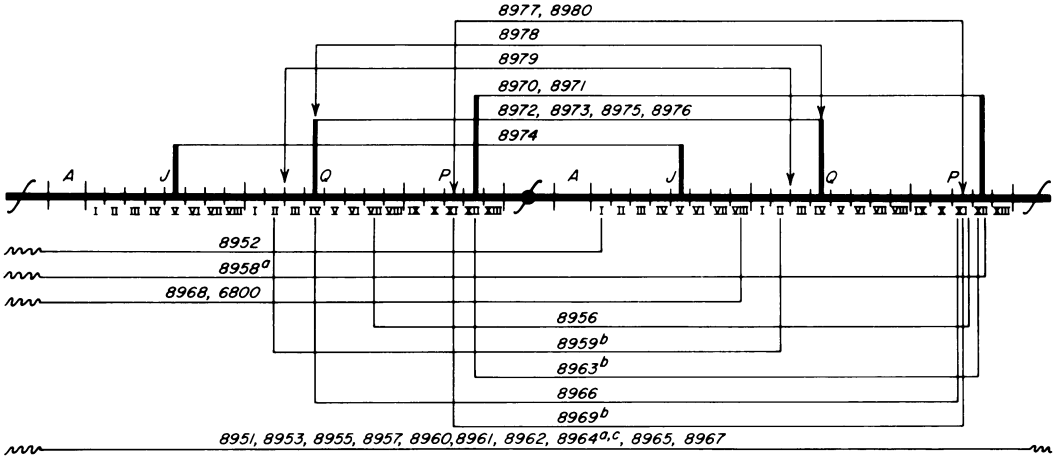

FIG. 8. *Genetic map of defects in HIPA-resistant mutants. The duplication-carrying chromosome was from either TA344 or TA3140; the mutations which these strains carry in this operon are not shown in this figure for simplicity. For all symbols, see legends to Fig. 1 and 3. The lines below the blunt line (chromosome) indicate the deletion. For the lines above the chromosome, see text for explanation. All numbers are allele numbers assigned to the HIPA-resistant mutations. (a) Covers ubiX. (b) Small deletion. (c) Deletes a gene to the right of hisP, responsible for fluoroacetate resistance (F. Ardeshir and G. Ferro-Luzzi Ames, unpublished data).*

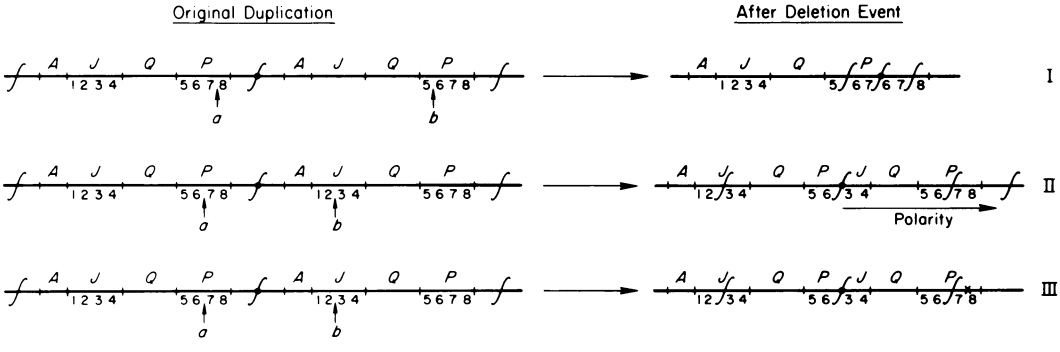

FIG. 9. *Model for the structure of deletion mutations still carrying duplications (TA3276 and TA3277). The numbers below the chromosome represent a hypothetical base sequence. The arrows (a and b) point to end points of deletion. The first line (I) shows a case where a hisP to hisP fusion has occurred; the second line (II) shows a hisP to hisJ fusion; the third line (III) shows a hisP to hisJ fusion. In all three cases, more than a full complement of the histidine transport operon is still present.*

(9). On the other hand, it is possible that these mutations are unstable point mutants occurring in a partially duplicated operon (Fig. 9, line III). At the moment we cannot distinguish between these possibilities.

DISCUSSION

We have presented evidence for the existence of duplications of the histidine transport operon among strains which have been selected for overproduction of the histidine transport components. We will not review in detail the criteria used for the claim that the mutations are duplications, because they all appear in an excellent recent review (9). In summary, we have demonstrated a gene dosage effect (increased J protein levels), the coexistence of two alleles of a single locus (two different J proteins produced; genetic demonstration of two alleles of either *hisQ* or *hisP*), instability of the mutation (both spontaneous and mutagen induced), mapping properties in agreement with the existence of a duplication join point near the histidine transport operon, and diploidy of transport genes (as suggested by the difficulty in isolating transport-negative mutants). By analogy with other systems (9), we assume that we are dealing with a tandem duplication, although we cannot exclude that some unduplicated material does not exist between the two copies of duplicated genes.

Below we discuss some of the properties of these particular duplications. If we follow the genealogy of the various duplication-carrying strains, we can assume that the duplications that they contain all have the same end points and join points because they were all derived by a sequence of transductional crosses from the same original duplication (present in TA344). We have the following information on the size and end points of this duplication. It does not carry the closest gene known, *ubiX*, which is located to the left of the promoter site, *dhuA*, and is about 80 to 90% linked to *dhuA* (6). It certainly covers *hisJ*, *hisQ*, and *hisP*, because we have evidence that the duplications can be heterozygotic for each of these genes. We cannot say with certainty that *dhuA* is duplicated. It is possible that the left end point is between *dhuA* and *hisJ* and has attached the transport operon to a new promoter, because the J protein apparently is produced in slightly higher amounts by one of the two operon copies; this would explain the approximately threefold increase in J level which the duplications exhibit (rather than the expected twofold). Another explanation for the higher than expected levels could be that, rather than a duplication, the strains actually carry "triplications" (or even higher numbers of copies [9]), thus raising the level of J to more than the expected double.

We have less information on the right-hand end point of the duplication due to the dearth of markers in that region. However, we do not think that the duplication is very large because we know that the site responsible for the elevated level of operon products (i.e., the second operon copy) is about 6% cotransducible with *purF*. Because we know that the left end is very close to *dhuA*, we can assume that the join point itself (i.e., the right end of the duplication) has approximately a 6% linkage to *purF*. This linkage corresponds to about 27 genes (11). The histidine transport operon is 35% linked to *purF* (corresponding to about 11 genes in the wild type). Therefore, the distance between the left end of the operon and the duplication join point (i.e., the size of the duplicated material) is approximately 16 genes. This relatively large distance is probably the site of frequent crossover events resulting in duplication loss, and thus it would account for the nonrecovery of some markers among the segregants arising from the instability of the duplication (as pictured in Fig. 7b).

Straus (21) and Hill and Combriato (10) demonstrated that the formation of tandem duplications of the regions including *glyS* and *glyT* is stimulated by a variety of mutagens which have different mechanisms of action. In both of these cases, the duplications arise by a *recA*-dependent process (10, 22). Here we also show that the loss of duplication (i.e., instability) is stimulated by a frameshift mutagen (ICR-191), although we have not tested any other mutagen in this respect. It is possible that the same mechanism is responsible for both the formation and the loss of tandem duplications. If this were true, it would imply that the same sites which are involved in determining the end points of the duplication are also involved in the recombination process resulting in duplication loss. Stimulation by mutagens of the *recA* activity at specific recombination sites may be involved in both the formation and the loss of duplications. On the other hand, any treatment which stimulates recombination might be responsible for an increased instability, without necessarily being related to the mechanism of duplication formation. More rigorous experiments, with a spectrum of mutagens followed by analysis of segregant genotypes, is needed to answer this question.

As discussed by Anderson and Roth (9), duplications can create an "operon fusion" in which genes from one operon can be fused to a different operon (or directly to a different promoter), thus

being put under a new functional control. In fact, one of our purposes in isolating duplication-carrying strains was to put the histidine transport operon under a new control and, therefore, raise the level of the gene products. The strains that we have studied have raised the level of J (and presumably of the other transport components) only two- to threefold. Thus, they are not particularly useful for the initial purpose, unless we find a way to tamper with the presumed control mechanisms and derepress production even further. Presumably, other duplications can be isolated which might place the operon under control of more efficient promoters. In this respect, we have preliminary evidence that selection to D-histidine growth always leads to a large number of duplications of the histidine transport operon. In fact, in an attempt at classifying the numerous papillae which appear on a D-histidine plate seeded with a histidine auxotroph, we observed that a large number were unstable for D-histidine growth, that the linkage of the mutation to *purF* was lower than 35%, and that the frequency of appearance of HIPA-resistant clones was greatly reduced (S. Kustu and G. Ferro-Luzzi Ames, unpublished data). Both of these properties are characteristic of duplication mutations. Also, the high frequency with which they arose and the fact that the selective pressure was exclusively for elevating the level of a limiting utilization component (i.e., transport) strongly suggest that they are duplication mutations.

One of the uses to which we put our duplication-carrying strains was in the isolation of deletion mutants. In the construction of a fine-structure genetic map for the histidine transport operon (6), the need arose for the availability of numerous deletions. As explained in the text, the elimination of both copies of *hisQ* and *hisP* in a duplication-carrying strain is an essential feature of the resistance to HIPA inhibition. In agreement with this, we found that at least 59% of all HIPA-resistant mutants arising in our selection were clearly deletion mutants. It is possible that an even higher percentage of deletions is formed. In fact, the 13 strains which are not clearly definable as deletion mutants could easily be such; the mapping behavior of 11 of them fits well with that expected for small deletions, and if they were point mutants they would have to have arisen through a double event (loss of duplication followed by a point mutation). The properties of the remaining two strains, which recombine with every mutant tested, can be explained most easily if we assume that they arose by a deletion of the original duplication but that they have deleted only part of it, still retaining a portion of it as a smaller duplicated region (Fig. 9). Thus, depending on what these 13 strains are, the percentage of deletion mutations recovered could be as high as 100%. More accurate mapping is necessary to resolve this point.

An analysis of the end points of the deletions thus generated indicates that they may be nonrandom. In fact, among the well-defined deletions (below the chromosomal line in Fig. 8) three (*his-8956*, *his8966*, and *his-8969*) end in the same region of *hisP* (XI); two (*his-8958* and *his-8963*) end in region XII of *hisP*; of these five strains, two (*his-8963* and *his-8969*) have both end points in the same homologous regions of each of the two copies (*hisP* XII and *hisP* XI, respectively). If we include the 13 strains which are not clear-cut deletion mutants in this analysis, the nonrandomness is even more striking: all of them start and end in the same region of each of the two copies, four of them appear identical (*his-8972*, *his-8973*, *his-8975* and *his-8976*), and one (*his-8978*) has end points in the same region (*hisP* IV) as the latter four. The clear-cut deletions also have end points which in some cases are in the same regions as those of the non-clear-cut deletions (see regions *hisP* IV, *hisP* XI, and *hisP* XII). It is not clear what this nonrandomness of deletion end points means. The end points of deletions previously isolated in strains not carrying duplications (6) seem to be randomly distributed. The present finding might indicate that the deletions presented in this paper arise by a different mechanism, which might be dependent upon the existence of a tandem duplication.

ACKNOWLEDGMENTS

This study was supported by Public Health Service grant AM12121 from the National Institute of Arthritis, Metabolism, and Digestive Diseases to G.F.-L.A.

We thank J. Roth, P. Anderson, F. Ardeshir, and D. Noel for helpful discussions.

LITERATURE CITED

1. **Ames, B. N., and H. J. Whitfield, Jr.** 1966. Frameshift mutagenesis in *Salmonella*. Cold Spring Harbor Symp. Quant. Biol. **31**:221–225.
2. **Ames, G. Ferro-Luzzi.** 1972. Components of histidine transport. Biological membranes, p. 409–426. *In* C. F. Fox (ed.), Membrane research. First ICN-UCLA Symposium on Molecular Biology. Academic Press Inc., New York.
3. **Ames, G. Ferro-Luzzi.** 1974. Resolution of bacterial protein by polyacrylamide gel electrophoresis on slabs. J. Biol. Chem. **249**:634–644.
4. **Ames, G. Ferro-Luzzi, and J. Lever.** 1970. Components of histidine transport: histidine-binding proteins and *hisP* protein. Proc. Natl. Acad. Sci. U.S.A. **66**: 1096–1103.
5. **Ames, G. Ferro-Luzzi, and K. Nikaido.** 1978. Identification of a membrane protein as a histidine transport

component in *S. typhimurium*. Proc. Natl. Acad. Sci. U.S.A. **75**:5447–5451.

6. **Ames, G. Ferro-Luzzi, K. D. Noel, H. Taber, E. N. Spudich, K. Nikaido, J. Afong, and F. Ardeshir.** 1977. Fine-structure map of the histidine transport genes in *Salmonella typhimurium*. J. Bacteriol. **129**: 1289–1297.
7. **Ames, G. Ferro-Luzzi, and J. R. Roth.** 1968. Histidine and aromatic permeases of *Salmonella typhimurium*. J. Bacteriol. **96**:1742–1749.
8. **Ames, G. Ferro-Luzzi, and E. N. Spudich.** 1976. Protein-protein interaction in transport: periplasmic histidine-binding protein J interacts with P protein. Proc. Natl. Acad. Sci. U.S.A. **73**:1877–1881.
9. **Anderson, R. P., and J. R. Roth.** 1977. Tandem genetic duplications in phage and bacteria. Annu. Rev. Microbiol. **31**:473–505.
10. **Hill, C. W., and G. Combriato.** 1973. Genetic duplications induced at very high frequency by ultraviolet irradiation in *E. coli*. Mol. Gen. Genet. **127**:197–214.
11. **Kemper, J.** 1974. Gene order and co-transduction in the *leu-ara-fol-pyrA* region of the *Samonella typhimurium* linkage map. J. Bacteriol. **117**:94–99.
12. **Kleckner, N., J. Roth, and D. Botstein.** 1977. Genetic engineering *in vivo* using translocatable drug-resistance elements: new methods in bacterial genetics. J. Mol. Biol. **116**:125–159.
13. **Krajewska-Grynkiewicz, D., W. Walczak, and T. Klopotowski.** 1971. Mutants of *Salmonella typhimurium* able to utilize D-histidine as a source of L-histidine. J. Bacteriol. **105**:28–37.
14. **Kustu, S. G., and G. Ferro-Luzzi Ames.** 1974. The histidine-binding protein J, a histidine transport component, has two different functional sites. J. Biol. Chem. **249**:6976–6983.
15. **Lever, J. E.** 1972. Purification and properities of a component of histidine transport in *Salmonella typhimurium*. The histidine-binding protein J. J. Biol. Chem. **247**:4317–4326.
16. **Noel, K. D., and G. Ferro-Luzzi Ames.** 1978. Evidence for a common mechanism for the insertion of the Tn10 transposon and for the generation of Tn10-stimulated deletions. Mol. Gen. Genet., in press.
17. **Roth, J. R.** 1970. Genetic techniques in studies of bacterial metabolism. Methods Enzymol. **17**:1–35.
18. **Sanderson, K. E., and P. E. Hartman.** 1978. Linkage map of *Salmonella typhimurium*, edition V. Microbiol. Rev. **42**:471–519.
19. **Schmieger, H.** 1971. A method for detection of phage mutants with altered transducing ability. Mol. Gen. Genet. **110**:378–381.
20. **Sletzinger, M., R. A. Firestone, D. F. Reinhold, C. S. Rooney, and W. H. Nicholson.** 1968. The α-hydrazino analog of histidine. J. Med. Pharmacol. Chem. **11**: 261–263.
21. **Straus, D. S.** 1974. Induction by mutagens of tandem gene duplications in the *glyS* region of the *E. coli* chromosome. Genetics **78**:823–830.
22. **Straus, D., and L. D'Ari Straus.** 1976. Large overlapping tandem genetic duplications in *S. typhimurium*. J. Mol. Biol. **103**:143–153.
23. **Vogel, H. J., and D. M. Bonner.** 1956. Acetylornithinase of *Escherichia coli*: partial purification and some properties. J. Biol. Chem. **218**:97–106.