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Giovanna Ferro-Luzzi Ames
University of Wisconsin - Madison

K. Dale Noel
Marquette University, dale.noel@marquette.edu

H. Taber
University of California - Berkeley

E. Negri Spudich
University of California - Berkeley

Kishiko Nikaido
University of Wisconsin - Madison

See next page for additional authors

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Authors
Giovanna Ferro-Luzzi Ames, K. Dale Noel, H. Taber, E. Negri Spudich, Kishiko Nikaido, J. Afong, and F. Ardeshir

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Fine-Structure Map of the Histidine Transport Genes in *Salmonella typhimurium*

G. FERRO-LUZZI AMES,* K. D. NOEL, H. TABER,[1] E. NEGRI SPUDICH, K. NIKAIDO, J. AFONG, AND F. ARDESHIR

Biochemistry Department, University of California, Berkeley, California 94720

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A fine-structure genetic map of the histidine transport region of the *Salmonella typhimurium* chromosome was constructed. Twenty-five deletion mutants were isolated and used for dividing the *hisJ* and *hisP* genes into 8 and 13 regions respectively. A total of 308 mutations, spontaneous and mutagen induced, have been placed in these regions by deletion mapping. The histidine transport operon is presumed to be constituted of genes *dhuA*, *hisJ*, and *hisP*, and the regulation of the *hisP* and *hisJ* genes by *dhuA* is discussed. The orientation of this operon relative to *purF* has been established by three-point crosses as being: *purF dhuA hisJ hisP*.

In *Salmonella typhimurium* histidine is transported by at least five transport systems (1). One of these is the high-affinity histidine permease. Two of the components of this system are the J protein (a periplasmic histidine-binding protein [3, 4]) and the P protein (characterized genetically and physiologically and presumed to be membrane bound [3]). These two proteins are coded for by genes *hisJ* and *hisP*, respectively, which are closely linked to each other and to a third site, *dhuA* (12), which apparently regulates the expression of the first two genes. Mutations in *dhuA* allow growth of a histidine auxotroph on D-histidine by elevating the level of D-histidine transport, which is limiting in D-histidine utilization (3, 12). Genes *dhuA*, *hisJ*, and *hisP* are presumed to constitute a histidine transport operon and are located at 75 min on the *Salmonella* chromosomal map, close to *purF* (5).

The isolation of mutants affecting the high-affinity histidine transport system has been one of the basic approaches in our research in histidine transport and has yielded extensive information. During the course of this work it became apparent that it was necessary to characterize genetically the large variety of mutants that were being generated in the *hisJ* and *hisP* genes.

In this communication we present a fine-structure genetic map of the genes in the histidine transport operon.

MATERIALS AND METHODS

Strains. All strains used are nonlysogenic derivatives of *S. typhimurium* LT2. Most strains mutated in the histidine transport genes have been isolated either from the wild type or from a histidine-requiring strain, TA831 (*hisF645*), or from TA271 (*dhuA1 hisF645*). Table 1 lists all of the strains that contain deletion mutations in the histidine transport operon. All of the other strains that contain nondeletion mutations in any of the histidine transport operon genes appear in the figures. The nomenclature for strains containing a *tet*^{r} element insertion is as follows (*in* Bukhari et al., ed., *DNA Insertions*, in press; J. Roth and K. Sanderson, personal communication): if the gene in which the insertion occurs is known, the gene gets an allele number as usual, followed by the symbol :: (11) and *tn10* (for the particular *tet*^{r} element used in this work [11]); thus the insertion in gene *hisF* in strain TT29 is called *hisF8539::tn10*. If the gene is unknown, the nomenclature is similar to that devised for conditional (temperature-sensitive [Ts] mutations [10], which uses the map position. The first letter will be *z* (for insertions of transopsons) and the second and third will indicate the position on the map (10) followed by an allele number, the symbol ::, and *tn10*; thus, the insertion near the transport genes at 75 min, in strain TA2099, is identified as *zhf102::tn10*.

Media. Cells were grown with vigorous aeration in nutrient broth (0.8% Difco-Bacto nutrient broth; 0.5% NaCl). Crosses were performed on minimal agar plates (15) to which D-histidine (10 μmol) or *N*-acetylhistidine (2 μmol) was added to each plate by spreading, just before use. Minimal agar and nutrient broth agar plates contained 25 μg of tetracycline-hydrochloride when selection for the tetracycline resistance element was necessary.

Isolation of mutations in the histidine transport operon. Mutants defective in the transport genes were isolated from TA271 (*dhuA1 hisF645*) either as resistant to inhibitory analogues that are transported by the histidine permease (14, 17) or by penicillin selection on D-histidine (3). The analogues used were α-hydrazino-imidazole propionic acid

[1] Present address: Department of Microbiology, School of Medicine and Dentistry, University of Rochester, Rochester, NY 14642.

TABLE 1. *Bacterial strains*

Strain	Deletion no.[a]	Genes affected by deletion mutation
TA2939	*5638*	*dhuA hisJ*
TA2944	*5643*	*dhuA hisJ*
TA1834	*5650*	*dhuA hisJ*
TA2918	*6776*	*hisJ*
TA3057	*6752*	*hisJ hisP*
TA3010	*6704*	*ubiX dhuA hisJ hisP*
TA3016	*6711*	*hisP*
TA3011	*6705*	*ubiX dhuA hisJ hisP*
TA3012	*6706*	*ubiX dhuA hisJ hisP*
TA3015	*6709*	*dhuA hisJ hisP*
TA3013	*6707*	*ubiX dhuA hisJ hisP*
TA3014	*6708*	*ubiX dhuA hisJ hisP*
TA3017	*6712*	*hisP*
TA3018	*6713*	*hisP*
TA1708	*5549*	*hisP*
TA1008	*5503*	*hisP*
TA1741	*5575*	*hisP*
TA1835	*5651*	*ubiX dhuA hisJ hisP*
TA2964	*6663*	*ubiX dhuA hisJ hisP*
TA3058	*6753*	*ubiX dhuA hisJ hisP*
TA3059	*6754*	*ubiX dhuA hisJ hisP*
TA3060	*6755*	*ubiX dhuA hisJ hisP*
TA1851	*5670*	*ubiX dhuA hisJ hisP*
TA3077	*6794*	*ubiX dhuA hisJ*

[a] All strains contain also the histidine deletion *hisF645*, except for TA1851, which is prototrophic.

(HIPA) (17) and O-diazoacetylserine (azaserine) (4), and the selection was performed either on minimal medium for prototrophic strains or in the presence of either the histidine precursor histidinol (100 μmol/plate) or the histidine derivative carnosine (1 μmol/plate) in strains auxotrophic for histidine. Each strain was purified twice, and its properties were verified by streaking each strain from the center of a petri plate outwards and placing the analogue or D-histidine in the center (radial streak test [15]). Each resistant strain isolated was derived from an individual culture originating from a single colony of the parental strain (TA271, in most cases). In cases when multiple strains were isolated from the same culture, they were crossed against each other, and those mutants which gave no recombination were considered identical and only one isolate was saved. Resistance to azaserine or nongrowth on D-histidine can arise as a consequence of mutations either in the *hisP* or the *hisJ* genes (3, 4, 14). On the other hand, resistance to HIPA arises as a consequence of mutations in *hisP* but not in *hisJ* (3), except for *hisJ* polar mutants. It should be noted that azaserine is a powerful mutagen, apparently causing base pair substitutions (B. N. Ames, personal communication). Thus, some of the mutants isolated in the presence of azaserine probably arose as a mutagenic event induced by azaserine.

For the mapping of mutations that had been obtained by analogue resistance in strains other than TA271 and that were prototrophic for histidine, a histidine requirement was introduced by either of the two following procedures. (i) The mutation was transduced into a background containing the *hisF645* mutation by preparing phage on the mutants, transducing TA1852 (*purF145 dhuA1 hisF645*) to purine independence on L-histidine, and then scoring the Pur$^+$ transductants for the presence of the transport mutation by the radial streak test. Only phage-free (phage-sensitive) clones were saved. (ii) A *hisF* mutation due to the insertion of a *tet*^{r} element (11) in the *hisF* gene (strain TT29, *hisF8539::tn10* obtained from J. Roth) was introduced in the prototrophic strains by transducing them to tetracycline resistance on a nutrient agar plate with phage prepared on TT29. A single tetracycline-resistant recombinant was purified and verified for being a histidine auxotroph and for phage sensitivity. This procedure is very simple, quick, and essentially failproof.

Enrichment for deletion mutations. The basis for this procedure is described in the text. Fosfomycin (0.08 ml of a 5-mg/ml solution of disodium fosfomycin; Merck & Co., Inc., Rahway, N.J.), 0.03 ml of 0.1 M carnosine, and 10^8 cells of strain TA271 were added to 2.0 ml of molten agar and overlaid on a minimal agar plate. The final concentration of fosfomycin in the agar was 13 μg/ml. Disks containing HIPA were placed on the agar surface, and the plates were incubated for 2 days at 37°C. Resistant clones were picked from the clear zones around the HIPA disks and streaked for single-colony isolation on nutrient broth agar. Those clones that grew as small colonies on nutrient broth and the growth of which was stimulated by *p*-hydroxybenzoic acid were tested for recombination with strains carrying point mutations in *hisJ* and *hisP*.

Transductions. Mutations in either the *hisJ* or *hisP* genes eliminate growth on D-histidine in *dhuA1*-containing strains. This provides the means of selecting for HisJ$^+$ or HisP$^+$ recombinants as colonies growing on D-histidine in crosses between histidine transport mutants. Thus, in a cross between *hisJ* or *hisP* mutants both strains are auxotrophic for histidine (carrying deletion mutation *hisF645*), one or both strains carry the mutation *dhuA1*, and the plate contains D-histidine. All transductions were performed with P22 phage *int4* (15) or with a high-transducing derivative of P22 phage (16) into which an *int* mutation was introduced (obtained from J. Roth). Phage was grown on the donor strain as described (15). Multiple crosses were performed by spreading a recipient bacterial strain on a D-histidine plate and then placing on it drops of about 0.05 ml of each donor phage preparation, containing approximately 10^{10} phage particles per ml (spot test). The plates were inspected for recombinants after incubation at 37°C for 2 days. In all cases of a negative result (no recombinants) the conditions were such that point mutations at the opposite ends of a gene would have yielded more than 5,000 recombinants. Such a negative result was often rechecked by performing a cross with a single pair on a whole plate, in which case two point mutants could yield as many as 50,000 recombinants. A Bertani box, a plastic chamber containing 25 individual wells for the phage suspensions, was also used in preliminary crosses among large numbers of mutants.

Crosses between two *dhuA*$^+$ *hisP* strains cannot

be performed on D-histidine. Such crosses were performed on *N*-acetylhistidine, provided a wild-type *hisJ* gene could also be recovered in the cross. Growth on *N*-acetylhistidine is completely dependent on the presence of an intact *hisP* gene, but does not require a *dhuA1*-type mutation. The dependence upon a wild-type *hisJ* gene is not complete: *hisJ* mutants grow poorly on *N*-acetylhistidine.

Insertion of *tet*^{r} element in the proximity of the histidine transport operon. A genetic element, *tn10*, responsible for tetracycline resistance (11) was transduced into the chromosome near the histidine transport genes to be used as a marker for three-point crosses necessary to define the order of these genes. Strain TA1852 (*purF145 dhuA1 hisF645*) was transduced to purine prototrophy (in the presence of histidine) with P22 phage prepared on a mixed culture of prototrophic bacterial colonies, each individually resistant to tetracycline because of the insertion of the *tet*^{r} element (11) in a different region of the chromosome (the above phage were supplied by J. Roth). The Pur$^+$ recombinants were replicated onto tetracycline-containing (25 μg/ml), nutrient agar plates to identify those that had simultaneously acquired the *tet*^{r} element. This indicates that the inserted element is cotransducible with *purF*. One of these recombinants, TA3088 (*dhuA1 zhf102::tn10 hisF645*), was found to have the *tet*^{r} element closely linked to the histidine transport genes (80%). TA3088 and a *hisP*-containing derivative, TA3098, were used for establishing the correct order of genes in that area of the chromosome.

Isolation of deletions by loss of the *tet*^{r} element. It has been shown (11) that excision of the *tet*^{r} element is inaccurate, often leading to the loss of neighboring chromosomal material and to the creation of deletions of a variety of sizes (J. Roth and D. Botstein, personal communication). If the *tet*^{r} element is inserted into the bacterial chromosome near, but not within, a gene of interest, deletions can be obtained in that gene by selecting for loss of gene function simultaneous to the loss of *tet*^{r} expression. In this manner we obtained deletions affecting the histidine transport genes by selecting for resistance to azaserine in strain TA3088 and screening the azaserine-resistant colonies for tetracycline sensitivity. Among 354 azaserine-resistant colonies, 63 were tetracycline sensitive and 32 of these contained deletions affecting histidine transport. All deletions generated from the *tet*^{r} element were slow growers on nutrient broth plates, indicating that the *tet*^{r} element in TA3088 lies between *ubiX* and *purF*. Of 19 independently derived deletions, 6 end in *hisJ*, 1 ends in *hisP*, and 12 extend beyond the *hisP* gene. Interestingly, all six independent deletions ending in *hisJ* appear to end at the same point in the *hisJ* gene. One of these, *his-6794*, is shown in Fig. 4.

Histidinol growth. This test distinguishes between a *hisP* mutant (no growth), a HisP$^+$ isogenic strain (poor growth), and an odd-group *hisP* mutant mapping in region IV (see Fig. 5; good growth, only near disk). The test is by radial streaking against 10 μmol of L-histidinol at 20°C and is valid for strains that carry the *hisF645* mutation. Not all histidine-requiring strains can be used in this test: mutants blocked in early steps in the histidine pathway (e.g., in *hisG*) grow much better on histidinol than do *hisF* mutants.

Sodium dodecyl sulfate-acrylamide gel electrophoresis. Sodium dodecyl sulfate-acrylamide gel electrophoresis was performed on a slab gel as described previously (2).

RESULTS AND DISCUSSION

Isolation and characteristics of deletion mutants in the histidine transport region. Strains carrying the following deletion mutations affecting the histidine transport genes were obtained: (i) deletions contained within either *hisJ* or *hisP*; (ii) deletions having one terminus within the transport operon and the other in the neighboring areas, either to the right or to the left of the operon; (iii) deletions covering the entire operon and extending into both neighboring areas.

Among the deletions that have a terminus in the area to the left of the operon, many exhibited a characteristic phenotype: (i) poor growth on nutrient broth agar; (ii) poor growth on minimal agar; (iii) growth on minimal agar stimulated by the ubiquinone precursor *p*-hydroxybenozic acid; (iv) partial resistance to growth inhibition by the antibiotics kanamycin, neomycin, gentamicin, and fosfomycin; (v) a low level of ubiquinone (approximately 25% of wild-type level) that is restored to normal by the addition of *p*-hydroxybenzoic acid (data obtained in deletion mutation *his-5670* by Jacob Bar-Tana; personal communication). This phenotype is ascribed to the deletion of one or more genes presumed to be involved in the biosynthesis of ubiquinone, which we will refer to as *ubiX*.

We attempted to enrich for deletion mutants having the above phenotype, with the expectation of finding among them some having the right terminus within the operon. A collection of such deletion mutants is useful for the unambiguous ordering of point mutations in the operon. The strategy was to isolate mutants that were simultaneosuly resistant to one of the above-mentioned antibiotics and to HIPA. Fosfomycin was used since the aminoglycosides appeared to be antagonistic to HIPA; fosfomycin is slightly synergistic to HIPA. The procedure used is described above.

Among the resistant colonies appearing in this selection, 16% (9/57) were found to carry deletions affecting the histidine transport genes, and only half of the deletions were of the small colony type, i.e., covering the *ubiX* gene(s). The frequency of deletions among HIPA-resistant mutants in an ordinary selection is 10% (6/61). Thus, if an enrichment was

obtained, it was minimal. However, by the above procedure, we obtained 15 deletions with all types of termini (see above). Other deletions were obtained by the standard isolation procedure.

General features of the histidine transport region. Figure 1 illustrates the location of the cluster of histidine transport genes at 75 min on the *Salmonella* chromosome. By phage P22 transduction, the cluster is closely linked to *purF* (35%) but unlinked to *glpT* (glycerophosphate transport). By phage P1 transduction (G. Ferro-Luzzi Ames and E. Negri Spudich, unpublished data) the following cotransduction frequencies have been obtained: *glpT* to *purF*, 1%; *glpT* to *hisW*, 70%; *hisP* to *hisW*, 3%. Therefore, these genes are tentatively put in the following order: *purF hisP hisW glpT*. Because of the preliminary nature of these data, only the better established and more relevant information is included in Fig. 1. The linkage of *purF* to *glpT* (with phage P1) is similar to that obtained for the same genes in *Escherichia coli* with the same phage (7).

Site *ubiX* (see above) is located between *purF* and *dhuA*. The order *ubiX dhuA hisJ hisP* is defined by deletion analysis (Fig. 1). Several deletions covering *dhuA* and part of *hisJ* have been isolated: shown are *5592* and *5638*. In these deletions the *hisP* gene is genetically intact, though not functioning. Deletion *6752*, on the other hand, contains an unaltered *dhuA* site and covers all of *hisP* and most of *hisJ*. Deletion *5575* covers all of the *hisP* gene and leaves the *dhuA* and *hisJ* genes intact (see below). Thus, the order *dhuA hisJ hisP* is inferred. Site *ubiX* is on the *dhuA* side as shown by the existence of several deletions covering *ubiX*, *dhuA*, and *hisJ* and terminating within *hisP*; shown in Fig. 1 are *6704* and *6708*.

The orientation of this group of genes relative to *purF* has been established by three-point crosses that made use of strains (TA3088 and TA3098) containing a *tet*^{r} element inserted near *purF*. The *tet*^{r} element in TA3098 (*dhuA1 zhf102::tn10 his P6756* [in region VI, Fig. 5] *hisF645*) was first shown to be 80% linked by transduction to *dhuA1* and to be located on the *dhuA* side of the transport operon. This is demonstrated by two pieces of evidence: (i) some of the deletions isolated from TA3098 extend through the *dhuA* region and the *hisJ* gene, but do not go into the *hisP* gene; (ii) the results of the following three-point crosses (Fig. 2). Phage grown on TA3098 was used to transduce strains TA1619 (*dhuA1 hisP5526* [in region II] *hisF645*) and TA1710 (*dhuA1 hisP5551* [in region XIII] *hisF645*) to growth on D-histidine. The recombinants were then checked for tetracycline resistance. The frequency of tetracycline-resistant colonies is higher among the recombinants obtained with TA1619 (8%; 4/50) than with TA1710 (<2%; 0/50), as would be expected if the *tet*^{r} element were located on the same side of *hisP6756* as *hisP5526*. Therefore, the *tet*^{r} element in TA3088 and TA3098 is proximal to *dhuA* and distal to *hisP*.

Next, we established by means of the following cross that the *tet*^{r} element is located between *purF* and *dhuA*, thus determining the orientation of the transport operon relative to *purF* (Fig. 3). Phage prepared on TA3088 was

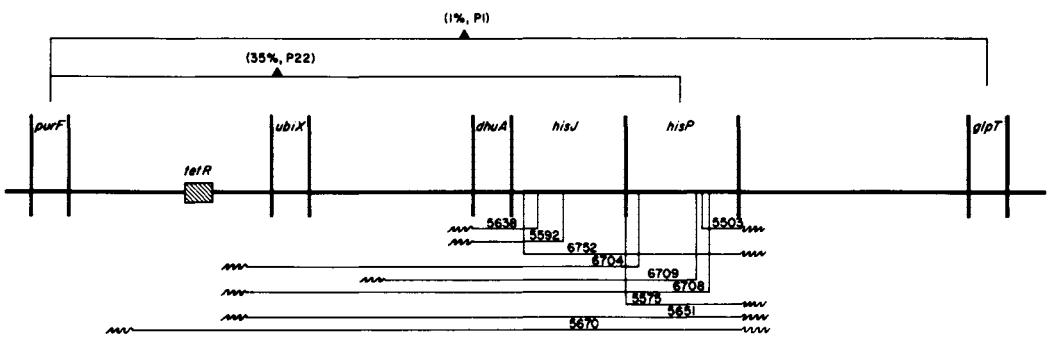

FIG. 1. *Location and order of the histidine transport genes in the Salmonella chromosome. The thicker horizontal line represents the chromosome. The thinner horizontal lines, below the chromosomal line, represent deletion mutations. The thick vertical lines delimit the genes. Thin vertical lines define the termini of a deletion and separate mutations that recombine with the deletion from those that do not. A wavy line indicates that the terminus of the deletion has not been genetically defined (usually because it falls in presently unmapped territory). Only some of the deletions are presented in this figure. Gene purF is located at 75 min. The numbers in parentheses indicate the linkage as obtained by phage cotransduction (P22 or P1) between those genes connected by the thin line above the chromosome. The rectangle between purF and ubiX represents the tet*^{r} *element, as located in strain TA3088.*

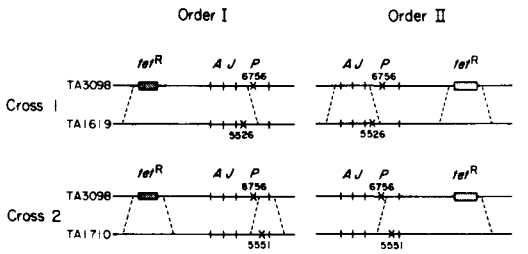

FIG. 2. *Crosses defining the order tet^r dhuA hisP. HisP^+ tet^r recombinants require a double crossover in cross 1 and a quadruple crossover in cross 2 if order I is correct. The opposite is true if order II is correct. Phage prepared on TA3098 was the donor (top line) in both crosses.*

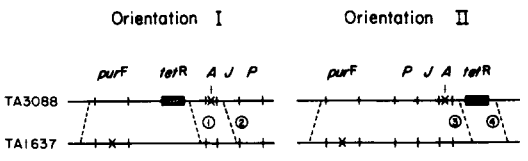

FIG. 3. *Cross defining the orientation of the histidine transport operon relative to purF. Phage prepared on TA3088 was the donor (top line). See text for details.*

used to transduce TA1637 (*purF145 hisF645*) to purine prototrophy on L-histidine. The Pur$^+$ recombinants were screened simultaneously for tetracycline resistance and growth on D-histidine. If the *tet^r* element in TA3088 is between *purF* and *dhuA1*, then essentially all recombinants that have become D-histidine growers (i.e., acquired the *dhuA1* mutation) will also have acquired the *tet^r* element (crossover 2 in Fig. 3). However, not all of the tetracycline-resistant recombinants will have acquired the *dhuA1* mutation (crossover 1 in Fig. 3). The opposite would be true if *dhuA* were between *purF* and the *tet^r* element (crossovers 3 and 4 in Fig. 3). Out of 136 Pur$^+$ recombinants, 29 (21%) were D-histidine growers, and all of them (100%) were tetracycline resistant. Of the 136 Pur$^+$ recombinants, 36 (27%) were tetracycline resistant and, of those, 29 (81%) were D-histidine growers. Thus, orientation I in Fig. 3 is established.

Figure 1 shows other deletions covering the whole of the *hisP* gene; most of them also cover the *hisJ* gene, the *dhuA* site, and *ubiX*. Deletion *5575* covers all *hisP* mutants tested and recombines with all *hisJ* mutants tested. Strains carrying *his-5575* produce J protein of unaltered molecular weight, indicating that the deletion has not entered the *hisJ* gene. Thus, its left end is somewhere between the end of the *hisJ* gene and the beginning of the *hisP* gene. The right end of deletion *5575* may terminate within the *hisP* gene or extend into the neighboring area. This mutation has been very useful for preliminary screening of all mutants of HisP$^-$ phenotype, allowing us to determine which ones have an intact *hisP* gene and therefore are located in the regulatory site (*dhuA*) or are polar *hisJ* mutants.

Among the large deletions, *5670* is presumed to extend far to the left toward *purF* because it has an increased linkage to *purF* (89% instead of 35%). Deletion *6709* is indicated as terminating short of *ubiX* because it does not have the poor growth phenotype typical of deletions covering *ubiX*, but it is resistant to kanamycin, whereas *his-5638* and *his-5592* are not. Thus we assume it covers an unknown gene located between the left termini of *his-5638* and *his-5592* and *ubiX*.

***dhuA* site.** Figure 4 illustrates the map of the *hisJ* gene and the *dhuA* site, with the neighboring region of the *hisP* gene. Our working hypothesis is that *dhuA* is an operator/promoter site in an operon composed of *hisJ* and *hisP* and that reading of this operon is from left to right as depicted in Fig. 1. We have shown previously (3, 4) that the *dhuA* site is involved in the regulation of the *hisJ* gene function: appropriate mutations in the *dhuA* site alter the level of the J protein. Mutations in *dhuA* are also thought to influence the functioning of the *hisP* gene (see below). In agreement with the operator/promoter hypothesis is the fact that mutation *dhuA1* (the most thoroughly analyzed of *dhuA* mutations) appears to act only in the *cis* position (see below).

***hisJ* gene.** A total of 59 spontaneous *hisJ* mutations have been mapped in the eight regions (Roman numerals) defined by six deletion termini (Fig. 4). On the basis of available evidence we cannot define precisely where the regulatory site ends and the structural gene starts. Region I is provisionally defined by the left terminus of deletion *6752* and the presumed beginning of the *hisJ* gene. None of the three mutants in region I produces J protein, as shown by sodium dodecyl sulfate-acrylamide gel electrophoresis, and they are all polar for P function. Thus, they may be either nonsense *hisJ* mutants or located in a regulatory region (*dhuA* or otherwise). Region II definitely covers a portion of the structural gene for J, because *hisJ5626*, which maps in this region, codes for a nonfunctional J protein (14).

A total of 29 *hisJ* frameshift mutations (induced with the frameshift mutagen ICR-191) have been mapped: 27 of them are in region VIII. Clustering of frameshift mutations is a common occurrence (9).

We have shown that the J protein probably interacts with the P protein during transport

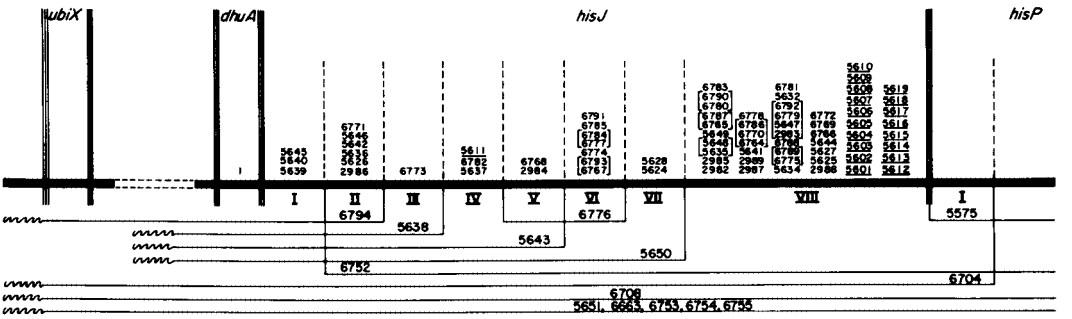

FIG. 4. *dhuA regulatory site and the hisJ gene. See the legend to Fig. 1 for an explanation of symbols. The Roman numerals refer to the various regions defined by the deletions. Each number above the chromosome represents a separate mutation. Underlined numbers are ICR-191-induced mutations. All mutations within each region have been crossed against each other in all possible combinations. Mutations that do not recombine with each other have been enclosed in vertical brackets and are presumed to have occurred at the same site or very close to each other. The order of mutations relative to one another within each region has not been established, and different columns of numbers do not imply different locations within the region. The thick vertical line separating dhuA from hisJ is arbitrary (see text).*

(6, 14). Thus, the J protein has two different sites, both essential for transport: the histidine-binding site and the interaction site. As part of that study we characterized a *hisJ* mutant (*5625*) that had a defect in the interaction site (6). This mutation is located in region VIII. More mutants of this type need to be isolated and mapped to define the genetic regions in *hisJ* that specify the structure of the interaction site.

Among the *hisJ* mutants isolated over the years, a number turned out to produce J proteins which exhibited altered mobility on sodium dodecyl sulfate-acrylamide gels. Usually, an altered mobility in such a system is indicative of an alteration in molecular weight. Mutant proteins have been detected with both increases and decreases (by different extents) in molecular weight. Two of these *hisJ* mutations have been mapped (*5625* and *5627*); they are located in region VIII. Not all available *hisJ* mutants have been screened for this property. We are presently analyzing the structure of some of these proteins to establish whether they have a truly altered molecular weight. We have previously discussed (6) the possibility that these proteins with altered molecular weights are the result of improper processing of a precursor of the J protein. A multiple map location of these mutations would be compatible with such a hypothesis, if processing occurs on a folded protein and if a protein mutated in any of a variety of sites is improperly folded.

***hisP* gene. (i) Fine-structure map.** Figure 5 illustrates the genetic map of the *hisP* gene and the adjacent region of the *hisJ* gene. Twelve deletions define 13 regions, indicated by the Roman numerals. A total of 195 spontaneous mutations have been mapped and are distributed fairly uniformly throughout the gene, except for a clear "hot spot" of a particular class of mutants in region IV (see below). Twenty-five *hisP* frameshift mutations (induced with ICR-191) were also mapped: as expected (9), they cluster in region II.

Temperature-sensitive mutants were sought, but never found. However, cold-sensitive *hisP* mutants occurred frequently (S. Govons Küstü, unpublished data). Five cold-sensitive mutations have been mapped: four of these (*5679, 5682, 5683,* and *5684*) map in the same site in region XI. The fifth one (*5678*) maps in region IV. We do not yet know, on the basis of such few mutations, whether there are preferred regions where cold sensitivity occurs.

As part of the demonstration that the J protein interacts directly with the P protein (6), first we have isolated a *hisJ* mutant that produces J protein with an altered interaction site and later a *hisP* mutant that produces a P protein capable of compensating for the J protein defect in the interaction site. The latter *hisP* mutation, *5700,* has been mapped in region XII. More mutants affecting the P protein interaction site need to be characterized before we will be able to define which regions of the *hisP* gene are involved in specifying the P interaction site.

The *hisP* gene consists of a single cistron, which exhibits some intragenic complementation (J. Lever, Ph.D. thesis, University of California, Berkeley, 1971).

(ii) Odd group. An interesting group of *hisP* mutations is the so-called "odd-group" in region IV. A large fraction of *hisP* mutations (27%) falls in this class, and they all map at the same

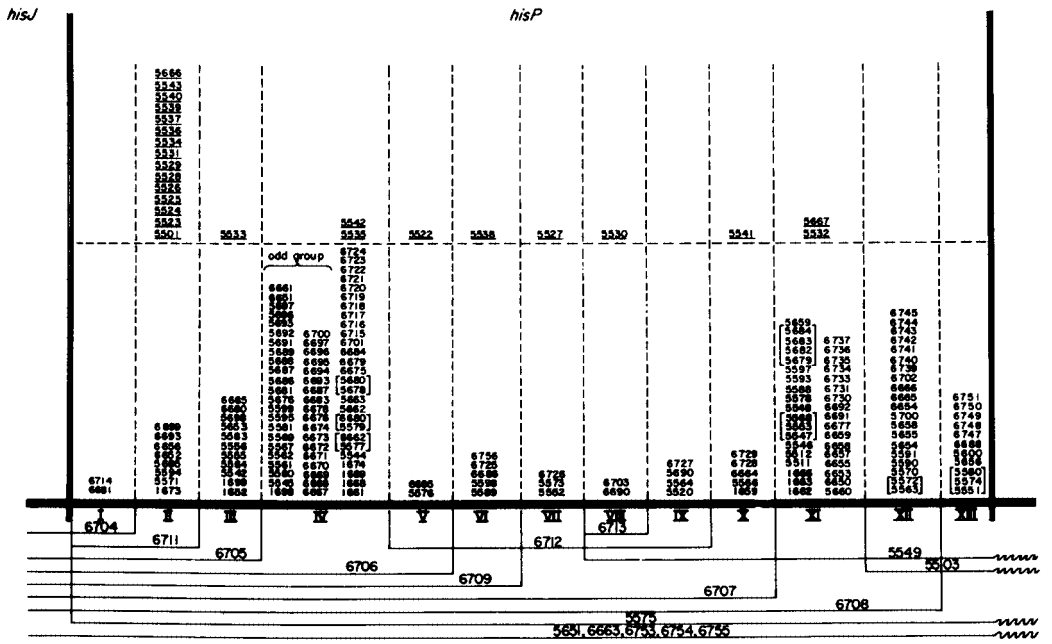

FIG. 5. *hisP gene. See the legends to Fig. 1 and 4 for an explanation of symbols. The mutations in each region have not necessarily been crossed with each other in all possible combinations. Thus, although vertical brackets do indicate non-recombination among the enclosed mutations, this does not exclude that other mutations from the same region will be found to map at the same site. The large group of mutations under the horizontal bracket in region IV, "odd group," includes mutations at a single site (see text); however, most of these mutations have been crossed only against one representative odd-group mutation and not against all others (see text). All ICR- 191-induced mutations (underlined) have been placed for convenience above the horizontal dashed line.*

site or extremely close together: no recombination was observed in crosses involving *5560*, *5562*, *5569*, and *5686* in all possible combinations and *5562* against *5545*, *5687*, *5689*, *5692*, and *5693*. A similar cross between recombining *hisP* mutants would yield up to a few thousand recombinants, depending on the distance between the *hisP* mutants. Most other odd-group mutations were placed in that class by their lack of recombination with *5560* (usually in a spot test) and by their ability to grow on histidinol (see below).

An additional property of odd-group mutants is that they are able to grow on histidinol (a histidine precursor, see above) even better than an HisP$^+$ strain, whereas all other *hisP* mutants are unable to grow on histidinol. The ability to grow on histidinol is caused by the same mutational event that causes the resistance to HIPA as shown by the following facts. (i) Recombinants that acquired the *hisP* mutation always acquired simultaneously the ability to grow on histidinol. (ii) Wild-type HisP$^+$ recombinants between an odd-group *hisP* mutant and a different *hisP* mutant always lost the ability to grow on histidinol. (iii) Revertants for the *hisP* mutation (selected on D-histidine) simultaneously became HIPA sensitive and lost the ability to grow on histidinol. Such revertants arise with low frequency (10^{-9}), and mutagen diethyl sulfate does not increase the reversion frequency.

Many of the mutants in the odd group had been exposed to histidinol and HIPA during their selection. The possibility that odd-group mutants arose in high frequency because of the selective pressure to grow on histidinol seems unlikely because reconstruction experiments to show improved growth of the histidinol fast growers as compared with other *hisP* mutants, when exposed to the same set of conditions used during isolation, failed to detect any difference. Moreover, several odd-group *hisP* mutants were obtained that had never been exposed to histidinol (as in His$^+$ strains or in His$^-$ strains grown on carnosine) or that had never been exposed to HIPA (arising during selection for azaserine resistance). None of these mutants was induced by the frameshift mutagen ICR-191.

At the moment we have no explanation for the properties of the odd-group mutants, and

they are under further investigation.

(iii) Regulation of *hisP*. The available evidence indicates that the *hisP* gene is under control of the *dhuA* region, which would act as an operator/promoter site. As we have no in vitro assay for the level of the P protein, the regulation of its synthesis by *dhuA* is inferred from the following observations. (i) Deletions (e.g., *5592*) that enter from the left and eliminate the *dhuA* site and part of *hisJ* without entering the *hisP* gene behave phenotypically like *hisP* mutants: i.e., they are HIPA resistant and do not grow on arginine as a nitrogen source (13) on histidinol (at 20°C) as a histidine source. (ii) Strains that contain the *dhuA1* mutation, which is known to elevate the level of the J protein about 10-fold, also might have an effect on the level of P protein, as indicated by a slightly improved growth on arginine as a nitrogen source (which does not need the presence of the J protein) (13) and on histidinol at 20°C.

Polarity. Several *hisJ* mutants exhibited a partial *hisP* phenotype, as measured by the size of the zone of inhibition caused by a disk containing 0.1 μg of HIPA. We feel that this phenomenon is due to a polar effect of the *hisJ* mutations. The following mutations in regions I and II have the strongest polar effect on the *hisP* gene, exhibiting a zone of inhibition with a diameter of less than 40% of that of the majority of the *hisJ* mutants: in region I, *5639*, *5640*, and *5645*; in region II, *5636*, *5642*, and *5646*. Mutation *5637* in region IV has a diameter of inhibition which is 80% of that of normal *hisJ* mutants. The weakest polar effect is among region VIII mutations: *5641*, *5644*, *5647*, and *5648*, which have a diameter of inhibition of 90%. Thus, the strongly polar mutations are concentrated in the left end of the *hisJ* gene; i.e., there is a gradient of polarity within this gene, similar to what has been shown in other cases for the first gene of an operon (8). Thus, on the basis of this limited evidence with the available polar mutations, we support the notion that the *hisJ* gene is read before the *hisP* gene and that they are both translated from the same messenger.

Dominance. Previous experiments (Lever, Ph.D. thesis) suggest that the *dhuA1* mutation is *cis* dominant with regard to the *hisP* gene. The experiments involved the construction of merozygotes (*S. typhimurium* chromosome and *E. coli* episome F'32) carrying appropriate mutations in *dhuA* and *hisP*. Growth on D-histidine (as evidence that a *dhuA* mutation is being effective) occurred only when the *dhuA1* chromosomal mutation was accompanied by a wild-type chromosomal *hisP* gene. Strain TA1512 (*his-2461 aroD5 dhuA1*/F'32 *hisP5506*) gave a zone of heavy growth, with a 4.5-cm diameter, around a disk containing 1 μmol of D-histidine, whereas strains TA1200 (*his-2461 aroD5 dhuA1 hisP1658*/F'32 HisP$^+$) and TA1786 (*his-2461 aroD5 purF145*/F'32 *dhuA504 hisP5507*) gave no growth or barely detectable growth. Appropriate control strains with other combinations of chromosomal and episomal transport mutations were built and tested. Therefore, the *dhuA1* mutation does not express its phenotype (i.e., it causes less than 10% of the expected growth on D-histidine) when combined with a functional *hisP* gene in *trans*. These experiments are not definitive because they have been done with hybrid genetic material from *S. typhimurium* and *E. coli*. Similar *cis-trans* tests performed on homologous merozygotes will be possible when an *S. typhimurium* episome carrying the transport genes is available.

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