A REFINED MAP OF THE *hisG* GENE OF *SALMONELLA TYPHIMURIUM*

I. HOPPE, H. M. JOHNSTON, D. BIEK AND J. R. ROTH

Department of Biology, University of Utah, Salt Lake City, Utah 84112

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ABSTRACT

The *hisG* gene is the most operator-proximal structural gene of the histidine operon; it encodes the feedback-inhibitable first enzyme of the biosynthetic pathway. Previously, *hisG* mutants were mapped into seven intervals defined by the available deletion mutations having endpoints in the *hisG* gene. The map has been refined using over 60 new deletion mutants. The new map divides the gene into 40 deletion intervals, which average approximately 30 base pairs in length. The map has been used to analyze the distribution of insertion sites for the transposable element Tn*10* and has permitted conclusions on the distribution of duplication endpoints. The map promises to be useful in analysis of *his* regulation and, more particularly, in the determination of the possible role of the *hisG* enzyme in this mechanism.

THE *hisG* gene is the most operator-proximal gene of the histidine operon (BRENNER and AMES 1971; HARTMAN, HARTMAN and STAHL 1971). It encodes the structure of the first enzyme in histidine biosynthesis, PR-ATP synthetase (BRENNER and AMES 1971). This enzyme has been purified (MARTIN 1963; WHITFIELD 1971; BLASI, ALOJ and GOLDBERGER 1971; VOLL, APELLA and MARTIN 1967; PARSONS and KOSHLAND 1974) and intensively analysed because of its sensitivity to feedback inhibition (MARTIN 1963; WHITFIELD 1971; MORTON and PARSONS 1977) and its complex subunit structure (PARSONS and KOSHLAND 1974a,b). Work of GOLDBERGER and coworkers has suggested that the enzyme may play a role in repression control of the histidine operon (GOLDBERGER 1974; DEELEY *et al.* 1975; MEYERS *et al.* 1975; MEYERS, LEVINTHAL and GOLDBERGER 1975; KLEEMAN and PARSONS 1977). To pursue a genetic analysis of this possibility, we devised a selection for deletion mutants of the *hisG* gene (SCOTT, ROTH and ARTZ 1975). It was found that deletion mutants lacking most of the *hisG* gene have normal operon regulation. Therefore, if the enzyme plays any role in *his* operon regulation, that role must be a subtle and dispensable one. It seems clear that further genetic analysis will be essential to final elucidation of *hisG* involvement in regulation. WAINSCOTT and FERRETTI (1978) have begun a genetic approach to identifying regions of the protein critical to substrate binding and feedback inhibition. This approach, like analysis of the *hisG* role in regulation, depends on high-resolution genetic mapping. To aid in approaching these problems, a revised and refined map of the *hisG* gene has been constructed. This paper describes isolation of deletions and construction of the genetic map. The

map is discussed in terms of its applications to the study of gene duplication, transposable genetic elements and the *his* operon control region. An accompanying paper (JOHNSTON and ROTH 1979) describes a positive selection for *hisG* mutations, which was used to select many new *hisG* mutants.

MATERIALS AND METHODS

Media: Nutrient broth (Difco) containing NaCl (0.5%) was used as rich medium for routine cell growth. Minimal medium was the E medium of VOGEL and BONNER (1956).

Strains: Previously described *hisG* point and deletion mutants were obtained from P. E. HARTMAN (HARTMAN, HARTMAN and STAHL 1971). Many new point mutants were isolated by means of a new selection method; this method and the mutants derived are described by JOHNSTON and ROTH (1979). Additional point mutants induced by proflavin are described in KOHNO and ROTH (1974). Strain TA81 (*his 01242 hisG6608*), which carries a strong polar mitomycin-induced *hisG* mutation (presumably a frameshift), was isolated by and obtained from JOYCE MCCANN. Strains TR3309 (*hisO+ hisG6608/F′ hisB2405*) and TR3057 (*hisO1242 hisG2101/F′ hisB2405*), used as parent strains for deletion isolation, were constructed by JOHN SCOTT. Some deletions isolated from TR3309 and all deletions from TR3057 were isolated by JOHN SCOTT (SCOTT, ROTH and ARTZ 1975; unpublished results).

Phage growth: Transductional crosses were mediated by a mutant of phage P22 (HT105/2) that transduces with high frequency (SCHMIEGER 1972). Overnight cultures (1 ml) of the donor strain were mixed with 5 ml of nutrient broth containing 0.4% glucose, full strength minimal salts (E salts), and 10^6 to 10^7 P22 particles per ml. The infected cultures were grown for five hrs and harvested by removing cell debris by a low speed centrifugation. Phage suspensions in growth medium were stored with a drop of chloroform at 4°.

Deletion selection method: Strain TR3309 or TR3057 were plated ($\sim 10^8$ cells per plate) on minimal medium containing 3-amino-1,2,4-triazole (AT; 15 m*M*), adenine (0.5 m*M*) and thiamine (0.05 m*M*). Resistant mutants were screened for possession of *hisG* deletion mutations. The rationale of this selection and the procedures for identification and recovery of deletion mutants are described by SCOTT, ROTH and ARTZ (1975).

Transductional mapping crosses: Mapping crosses were performed on minimal medium containing a low concentration (0.005 m*M*) of histidine. Except for the small *hisG-hisD* deletions isolated by INO *et al.* (1975), cells (2×10^8) and donor phage (5×10^9) were added directly to the selective plate and spread together. Since the small *hisG-hisD* deletions are somewhat leaky, crosses involving them were performed on unsupplemented minimal medium. Plates for all mapping crosses were incubated at 37° and scored after two to five days. These standard conditions yield approximately 15,000 recombinants per plate when donor phage is grown on wild-type LT2 and a *his* point mutant or small deletion is used as recipient. To make the final test for recombination more sensitive, five to ten plates were scored. Thus, the resolution of the map is based on crosses that would detect about 10^{-5}-fold reduction in recombination frequency as compared to an unrestricted cross (wild-type donor).

RESULTS AND DISCUSSION

The deletion selection method: Over 50 of the new deletions used to map *hisG* were obtained by a selection technique that demanded removal of a strongly polar *hisG* mutation. The selection demanded that the *hisB* gene remain intact and be either constitutive or derepressible to a high level. In effect, this restricted deletions to the *hisG*, *hisD* and *hisC* genes. Deletions removing the *his* control region could be recovered only if they fused the remaining *his* genes (including *hisB*) to a promoter that functions at a rather high level.

The parental strain (TR3309) in which deletion mutants were selected is diagrammed in Figure 1. This strain is mutant *hisG6608* carrying an *E. coli* F′ *his* episome; the F′ episome includes a complete *his* region with a mutation in the *hisB* gene. The *hisG6608* mutation has a highly polar effect on expression of genes located operator-distal. The merodiploid strain is phenotypically His$^+$ since the chromosomal *hisG* mutation and the episomal *hisB* mutation complement. Although the strain is prototrophic, it grows with very low levels of *hisB* enzyme activity. Therefore, growth of this strain is strongly inhibited by the histidine analogue aminotriazole (AT), which inhibits the *hisB* activity, IGP dehydratase (Hilton, Kearney and Ames 1965). When AT-resistant mutants are selected, one is selecting for an increase in *hisB* activity. This can occur by deletion of the *hisG6608* site if the deletion is not strongly polar and does not extend into the *hisB* gene. Other sorts of mutants that might be recovered are true revertants of *hisG6608*, true revertants of the episomal *hisB* mutation, polarity suppressor mutants (Beckwith 1963; Ratner 1976) or mutants that have impaired ability to take up AT. Since the F′ episome is of *E. coli* origin, low sequence homology restricts recombination with the Salmonella chromosome. Recombination between the *E. coli* and Salmonella *his* regions has never been detected (Fink and Roth, unpublished results). A second set of mutants was selected by the same procedure, using *hisG2101* as the strongly polar chromosomal mutation. Both sets of deletions are presented in Figure 2.

Map construction: The map (Figure 2) presents the results of transductional crosses between deletion mutants (used as recipients) and point mutants (used as donors). Under standard conditions (see MATERIALS AND METHODS) one can obtain over 15,000 *his*$^+$ transductants per plate in a cross between a *his*$^-$ point mutant and a wild-type donor. In the mapping crosses, a negative response represents no recombinants on at least five plates or more than a 10^5-fold reduction in recombination.

Deletions with "holes": At several points in the map, it was impossible to assign an order of point mutants that would account for all the recombination data. An order was chosen that accounted for most crosses. In each case, this left

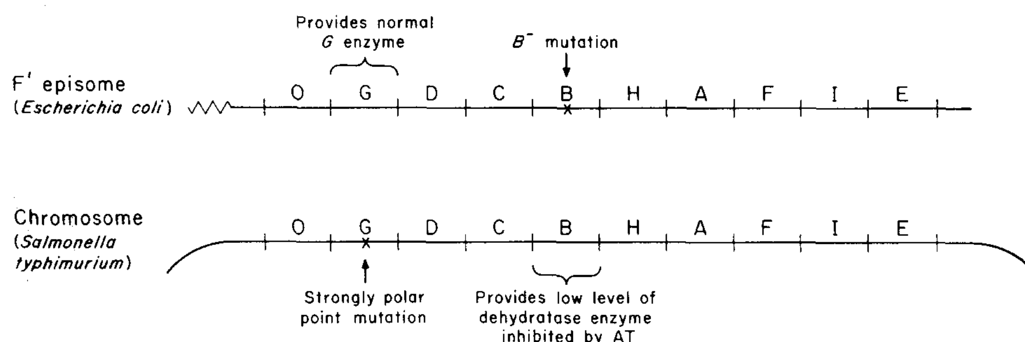


Figure 1.—Genotype of strain TR3309 in which *hisG* deletions were selected. The histidine analogue AT inhibits the *hisB* activity. The *hisG6608* mutation is extremely polar. Deletion of the site of the polar mutations allows an increase in *hisB* enzyme levels and thus confers AT-resistance on the strain.

FIGURE 2.—Deletion map of the *hisG* gene. Deletions are grouped according to their origin. For duplictaions (pi-numbers) the horizontal line indicates the extent of material included in the duplication. Point mutations are presented above the heavy horizontal line. Where available, the type of mutation is indicated (f = frameshift, n = nonsense, o = ochre, a = amber, u = UGA). Sites at which Tn*10* insertions have been identified are designated merely "Tn*10*." At the left-hand site, 17 insertion mutations have been mapped: *hisG8540*, *8543*, *8547*, *8549–8551*, *8553*, *8565–8570*, *8572*, *8575*, *9425*, and *9429*. At the right-hand site, two Tn*10* insertions have been mapped: *his G9433* and *hisG9436*.

a "problem" deletion that failed to recombine with a noncontiguous array of point mutations (the intervening positive responses are indicated by a dashed line in Figure 2.). In most cases, the problems involve only a few point mutations lying very near deletion endpoints. The map order in these problem regions is uncertain since a subjective decision was made to minimize contradictions. Several explanations of this phenomenon are possible: (1) *Marker effects on recombination.* Particular point mutations may be outside of a given deletion but fail to show recombination due to an alteration of DNA sequence that impairs recombination ability. Examples of this have been demonstrated in the *lacI* gene (SCHMEISSNER, GANEM and MILLER 1977). If this explanation is correct, crosses of higher sensitivity might resolve some of these problems. In one of the above problem regions, the sites that fail to recombine are Tn*10* insertion mutations. It is easy to imagine that such an insertion (10kb in length) might show impaired recombination with a nearby deletion mutation. (2) *Clonal selection in deletion isolation.* The deletion selection method (described above) demands removal of a polar mutation (*hisG6608*). If this strongly polar site were removed by a deletion that itself had a slight polar effect due to reading frame problems, a slow-growing (partially AT-resistant) clone might then arise. This clone would be under strong selection to acquire a frameshift mutation that would rephase the deletion and remove the polar effect. If this happened, one might occasionally encounter deletions associated with satellite frameshift mutations. (3) *Near homology at deletion endpoints.* In mapping of the T4 *rII* region, several deletions with associated point mutations were encountered (BARNETT *et al.* 1967). The explanation suggested was that deletions might arise by recombination between sequences of near homology. This would generate a new sequence in addition to the loss of material. This new sequence might show complicated patterns of recombination with nearby point mutations. This pattern might be indistinguishable from the pattern expected for a deletion with an associated point mutation.

Distribution of deletion endpoints: The deletions obtained from the positive selection method are generally as expected. All deletions that relieve the polar block of mutation *hisG6608* do, in fact, delete that site. Similarly the group of mutants selected from *hisG2101* remove that region of the map (see Figure 2). One exceptional deletion (*his-8444*) was isolated as a nonpolar derivative of *hisG2101*; the deletion does not remove the *hisG2101* site. It is not clear how this mutation satisfied the demands of the selection method.

Four types of deletions having both ends within the *hisG* gene have been isolated repeatedly. One type (*his-8500*) has recurred eight times and another (*his-8501*) five times. Two other types (*his-8495* and *his-8496*) have occurred two and three times, respectively. All other new deletion mutations are unique. This suggests that the repeats (especially the two types encountered most often) are deletion "hot spots." These sites may reflect repeated base sequences in the gene. Sixteen deletions, including the two most frequent classes, share a common endpoint slightly to the left of *hisG6608*. This site could include a sequence conducive to deletion formation.

The size distribution of deletions was unexpected. Although deletions ending in the *hisD* or *hisC* genes would have been detected, 50 of 53 deletions had their right endpoint within the *hisG* gene. This suggests the preferential occurrence of short deletions.

To the extent that deletion endpoints are random, some inferences can be made about the structure of the control region. Ten deletions have a left endpoint between the histidine attenuator site (defined by mutation *hisO1242*) and the left-most point mutation of the *hisG* gene. Only one deletion ends between the *his* promotor (defined by the *hisO2321* mutation) and the attenuator. This suggests that a rather large distance may separate the attenuator from the first coding sequences of the *hisG* gene.

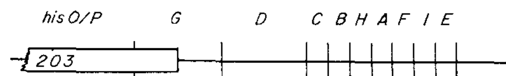
A portion of the hisG *gene essential to feedback sensitivity but dispensable for activity*: One set of *hisG* deletions (see Figure 2) was isolated by INO *et al.* (1975) as $HisD^+$ revertants of a *hisD* frameshift mutation mapping at the operator-proximal end of the *hisD* gene (*hisD497*). Deletions that remove the frameshift site can restore *hisD* activity. Some of these deletions extend into the *hisG* gene and presumably cause formation of a fused *hisG-hisD* protein. All of these deletions show weak growth on minimal medium and revert to become His^+ and feedback resistant (INO *et al.* 1975). The second-site mutations that permit the *hisG-hisD* protein to regain *hisG* function were found to map within the *his* region (probably within the *hisG* gene). Thus it seems that a catalytically active *hisG* enzyme can be formed that lacks sequences normally present at the carboxy-terminus of the wild-type enzyme. Since all such $hisG^+$ revertants seem to lack feedback sensitivity, it seems likely that this sequence is required for feedback inhibition. It is interesting to note that many of the point mutations causing feedback resistance or feedback hypersensitivity also map in this C-terminal portion of the genetic map (SHEPPARD 1964; HARTMAN, HARTMAN and STAHL 1971; ST. PIERRE 1968; O'DONOVAN and INGRAHAM 1965; WAINSCOTT and FERRETTI 1978). Our deletion mapping helps define the extent of this sequence. The longest deletions of this type lack five deletion intervals. If these intervals are of average size, as many as 50 amino acid residues may be involved in this dispensable portion of the protein.

*Nonrandom distribution of Tn*10 *insertion sites*: Mutations caused by insertion of the transposable element Tn*10* were first demonstrated by KLECKNER *et al.* (1975). These mutants have a defect due to interruption of the linear continuity of the gene in which insertion occurs; the mutants are resistant to tetracycline by virtue of genes carried by the transposable element. A large number of insertion mutants of the *his* operon have been selected both to investigate the nature of the transposable element and as a tool in genetic manipulations of the *his* operon (KLECKNER *et al.* 1975; KLECKNER, ROTH and BOTSTEIN 1977). The map distribution of Tn*10* insertions in the *his* operon is highly nonrandom. This can be seen from the distribution of Tn*10* insertions in the *hisG* gene (Figure 2). We have mapped 19 *hisG*::Tn*10* mutations, some isolated in this lab and some obtained from NANCY KLECKNER. Most of the mutants (17 of 19) fall within a single deletion interval. The other two mutants map within a second deletion

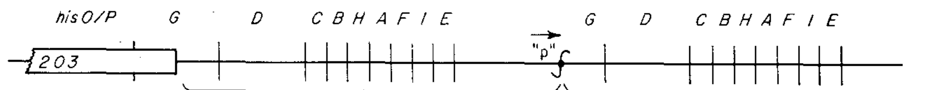
interval. Tn*10* insertions mapping in either deletion interval fail to recombine with others in the same interval. NANCY KLECKNER and coworkers (in preparation) have mapped additional insertion mutations in *hisG* and in other *his* genes and have demonstrated clearly that the Tn*10* element inserts preferentially at particular sites in the chromosome.

Distribution of duplication endpoints: A large number of tandem duplications with one endpoint in the *hisG* gene have been isolated and described previously (ANDERSON and ROTH 1978). The selection method for these duplications demanded that the *hisD* gene be fused to a foreign promoter and that the exchange occur at the operator-proximal side of the *hisD* gene. The general structure of these duplications is depicted in Figures 3A, 3B. If the *his* operon of the left-hand copy is replaced by a deletion mutation that removes all of the *hisG* gene, then the second copy carries the only *hisG* material present. The right-hand *his* operon in each duplication mutant carries, in effect, a *hisG* deletion mutation that removes material from the operator-proximal site of the *hisG* gene. If used as a donor in transductional crosses, the extent of the remaining *hisG* material can be mapped. The endpoint marks the position in the *hisG* gene at which an illegitimate exchange occurred with a distant point on the chromosome to generate a tandem duplication. The positions of these breakpoints for duplications isolated in rec^+ and rec^- backgrounds are presented in Figure 2. All duplications isolated in a rec^+ background end between the *hisD* and *hisG* genes. Many of the duplications isolated in a rec^- background also end in this intergene spacer, but others end within the *hisG* gene. The mapping of duplication endpoints and the significance of their distribution are discussed elsewhere (ANDERSON and

A. Promoter deletion *his-203*



B. Duplication, HisD$^+$ revertant of *his-203*



C. Duplication with deletion of left copy of *hisG*

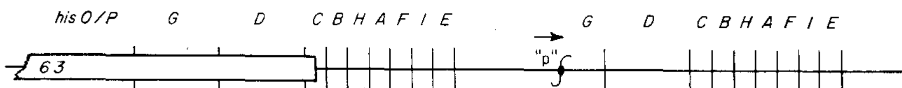


FIGURE 3.—(A.) Map position of deletion *his203*, which removes sequences essential to expression of the *hisD* gene. (B.) Structure of duplications obtained by selection for expression of the *hisD* gene of mutant *his203*. The *hisD* gene is placed under control of an unknown promoter. (C.) Strain used for mapping duplication end points. Deletion *his63* is crossed into the left copy of the *his* region. Only the right copy of the operon carries *hisG* material (adjacent to the join point). This strain can be used as donor in crosses to map the duplication endpoint. Construction of this strain has been described previously (ANDERSON and ROTH 1978).

ROTH 1977; ANDERSON and ROTH 1978). The data are presented here for comparison with deletion endpoints. It is apparent from the map (Figure 2) that duplications do not tend to occur at the sites that are hot spots for deletion formation.

Revertants of a promoter deletion: Deletion mutant *his-203* lacks the entire control region and part of the *hisG* gene. Due to lack of a promoter, this deletion mutant is unable to express the structurally normal *hisD* gene, which is located adjacent to *hisG*. AMES, HARTMAN and JACOB (1963) have described this deletion and a series of revertants selected for regaining *hisD* function. Many of these revertants carry extensions of the original deletion that remove more *hisG* material at one end and presumably fuse the operon to an unknown promoter by extending the other end of the deletion (*i.e.*, *hisG1300–1304*). Some mutants were described in which the extension within *hisG* could not be detected; it was not clear how *hisD* function had been restored. These mutants can now be shown to carry slight extensions of the parental *his-203* deletion (see deletions 1318, 1341, 1369, 1374, 1380, 1389 and 1618 in Figure 2).

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