

Transcription Initiation in the Histidine Operon of *Salmonella typhimurium**

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Abstract. Evidence is presented for two natural transcription initiators positioned internally in the histidine operon of *Salmonella typhimurium*. They were detected using highly polar *S. typhimurium his* mutants as recipients for F' *Escherichia coli his* episomes. These intergeneric complementation tests provide a sensitive method for the detection of other initiators and of terminators.

That at least part of the operator for the *his* operon lies distal to the transcription initiator is suggested by transduction analysis of a new highly polar mutant described in this paper.

The *his* operon of *S. typhimurium* consists of an operator region followed by nine genes encoding the enzymes for histidine biosynthesis.¹ Generally, deprivation of histidine from growing cultures results in a sequential expression of the genes which proceeds from the operator end and which yields a coordinate elevation of the *his* enzymes.¹ Repression of such derepressed cells also proceeds sequentially² and is coordinate.¹ An alteration to the pattern of derepression yielding simultaneous but still apparently coordinate effects may be obtained by increasing the pool of one-carbon fragments in these cells.³ These data, the direction of polarity effects among *his* mutants,^{1, 4} and studies of the size of *his* mRNA,^{1, 5} suggest this operon is transcribed into a single species of polycistronic mRNA. A single promoter at the operator end is presumed to serve as transcription initiator.

Several deletion mutants have been reported which lack both *hisO* and one or more of the proximal structural genes.¹ Some of these are highly polar and have been interpreted as lacking the *his* promoter. Our isolation of a new polar mutant, *his-3601*, led us to re-examine the properties of such mutants. We report evidence for the existence of two transcription initiators positioned internally in the *his* operon. These initiators are normally present, but may have insignificant expression in the normal system. In addition to the natural transcription initiators, mutationally derived initiators whose level of function are not determined have been obtained in the first structural gene.

Detection of the initiators makes use of complementation with appropriate *E. coli* F' *his* mutants. This intergeneric complementation occurs in the virtual absence of recombination, and is suggested as a general method for the detection of initiators and terminators.

Mapping of *his-3601* indicates that the *his* operon promoter, P₁, lies proximal to at least part of the *his* operator.

Materials and Methods. With the exceptions given below, media and bacteriological methods were essentially as described previously.⁶⁻⁹ Transductions for phage-sensitive recombinants were done using the integration deficient variant of P22, L4.¹⁰ *His-3601* is a spontaneously arising mutant isolated after mutant enrichment by recycling in penicillin. It was selected as a histidinol nonutilizing mutant in a strain containing a constitutive mutation, *hisO1242*, and amber mutation, *hisG2187*. The resultant strain is termed SC209. A variant of SC209 containing an amber suppressor for mutation *hisG2187* was constructed by first screening for *pro*⁻. This was then mated as recipient for F'*pro*⁺ *lac*⁻ (amber) for growth in the absence of proline. Phage L4 was propagated on a strain containing an amber suppressor for *hisG2187*; the episome-containing strain was then transduced for utilization of lactose and finally was cured of its episome. Designation and properties of these strains appear in Table 1. Other *Salmonella* strains are

TABLE 1. *Strains containing mutation his-3601.*

SC209	<i>his-3601 hisO1242 hisG2187</i>
SC211	<i>his-3601 hisO1242 hisG2187 pro</i> ⁻ /F' <i>pro</i> ⁺ <i>lac</i> ⁻ amber
SC215	<i>his-3601 hisO1242 hisG2187 pro</i> ⁻ <i>sup-723</i> /F' <i>pro</i> ⁺ <i>lac</i> ⁻ amber
SC278	<i>his-3601 hisO1242 hisG2187 pro</i> ⁻ <i>sup-723</i>

described elsewhere: *his* mutants,^{6, 11, 12} F'*lac*⁻ strains,¹³ F' T80 *his*⁺ *gnd*⁺ (*gnd* indicates the gene for expression of 6-phosphogluconate dehydrogenase) originally indicated to be an episome bearing *Salmonella* genes, was subsequently recognized as containing *E. coli* genetic information.¹⁴ Episomal mutants other than *E. coli hisF*⁻ and *hisC*⁻ were obtained through the courtesy of J. Roth and colleagues. Additional auxotrophic markers where required were obtained after penicillin screening following treatment with either *N*-methyl-*N'*-nitro-*N*-nitrosoguanidine or diethylsulfate. With two exceptions, episomes were established in donor strains containing chromosomal deletions of the relevant *his* genes. This avoided the possibility of growth in subsequent tests as a result of mobilization of *his*⁺ DNA from the chromosome of cells donating the F' factor. F'*hisA*⁻ was used as obtained in a background of *trp*⁻, *pur*⁻, *his-612*. F'*hisDa* was maintained by its *gnd*⁺ property in a background of *his-660* which had been mutated to lack gluconate utilization. F'*hisDb* and F'*hisDab* were used as obtained in a background of *trp*⁻, *pur*⁻, *his-612*. All other episomes were established in strains bearing *his-644*, *trpABE130*.

For all episomal tests apart from F'*hisD*⁻, episomes were established in the recipients by growth upon plates containing histidinol as a histidine source; purified clones were then streaked for isolated colonies on minimal media. The same results were obtained by plating donor strains directly onto lawns of the recipients using minimal plates. In tests using F'*hisD*⁻ mutants the donor strains were plated directly on lawns of the recipients. Enzyme assays of histidinol phosphate phosphatase were performed as described by Ames, Garry, and Herzenberg¹⁵ in the presence of 10⁻² M Mg⁺⁺ following a suggestion of B. N. Ames. The enzyme "isomerase" was assayed as described by Margolies and Goldberger.¹⁶ Protein determinations were by the Lowry method as described in reference 15.

Results. Intergeneric complementation: In a number of systems, episomal tests of intraspecies complementation yield positive results within 24 hours; low-level complementation may be obscured by the appearance of recombinant clones within two days, e.g., reference 17. Available data indicate the *his* operons in *S. typhimurium* and *E. coli* may have the same gene sequence,¹² but recombination in intergeneric crosses is rare,¹⁸ including crosses in the *his* region.^{14, 19} This is presumably a consequence of an underlying microheterogeneity of nucleotide sequences in the DNA of these organisms.¹⁸ Stable, hybrid partial diploids thus provide a convenient complementation system without significant possibility of recombination. In this procedure F'*his* factors bearing appropriate *E. coli his*

mutations are introduced into the *Salmonella his* auxotroph. Plating upon histidine-deficient media provides a sensitive test for even low levels of expression of the histidine biosynthetic enzymes.

The *Salmonella his* mutations used in these tests are included in Figure 1. Matings were conducted as detailed in *Materials and Methods*; results appear in Table 2. As in conventional tests, complementation using an $F'his^+$ factor yielded growth within 24 hours. Specific patterns of complementation are observed in tests using $F'his^-$ factors. In many cases this complementation is at a relatively low level, being revealed upon 48-hour incubation. Negative tests revealed no growth after incubation for six days. Mutant *his-3601* prevents expression of *hisG*, *hisD*, and *hisC* while clearly permitting expression of more

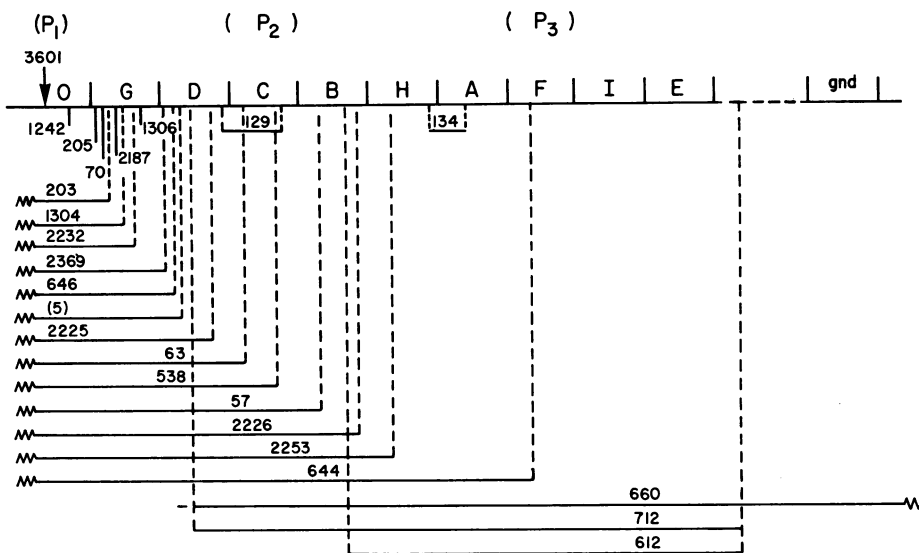


FIG. 1.—Simplified map of the histidine region in *S. typhimurium*. Any mutants involving only structural genes other than *hisD* can characteristically use the *hisD* enzyme product, histidinol dehydrogenase, and grow on supplied histidinol. Horizontal bar designated "(5)" represents the map position of mutants 2228, 2233, 2234, 2235, and 2237.

distal genes in the presence of the appropriate episomal *his^-* mutants. Both *hisOG203* and *hisOG2232* are polar in that they will not utilize histidinol as a source of histidine. Both fail to complement for *hisD* or *hisC* using episomes but do complement for expression of *hisB* and the other distal genes. Likewise, all eight *his OGD* mutants fail to complement for $F'hisC^-$ but will permit expression of more distal genes.

From these data, the mutants cited show polar effects preventing expression of contiguous, intact genes while allowing complementation for certain sets of distal ones. Comparable patterns are seen using *hisOGDCB57* and *hisOGDCBH2253*. Each of these strains complements for expression of *hisI* and *hisE* but each fails to express one or more complete genes adjacent to the end of the deletion.

Alternative explanations for these findings have been considered. Considering

TABLE 2. Growth by complementation-involving episomes.

Recipient <i>S. typhimurium</i>	Donor <i>E. coli</i> F' <i>his</i> ^a									
	<i>his</i> ⁺	<i>hisG</i> ⁻	<i>hisD</i> ^{-b}	<i>hisC</i> ⁻	<i>hisB</i> ^{-c}	<i>HAFIE</i> ⁻	<i>hisA</i> ⁻	<i>hisF</i> ⁻	<i>hisI</i> ⁻	<i>hisE</i> ⁻
SC278 (<i>pro</i> ⁻)	++	-	-	-	+	+	+	+	++	+
<i>hisOG203</i> , <i>arg</i> ⁻	++	-	-	-	+	+	+	+	++	+
<i>hisOG2232</i>	++	-	-	-	+	+	+	+	++	+
<i>hisOGD2369</i>	++	-	-	-	+	+	+	+	++	+
<i>hisOGD646</i>	++	-	-	-	+	+	+	+	++	+
<i>hisOGD2233</i> , <i>leu</i> ^{-d}	++	-	-	-	+	+	+	+	++	+
<i>hisOGD2225</i>	++	-	-	-	+	+	+	+	++	+
<i>hisOGDCB57</i> <i>aro</i> ^{-e}	++	-	-	-	-	-	-	-	++	+
<i>hisOGDCB2226</i>	++	-	-	-	-	+	+	+	++	+
<i>hisOGDCBH2253</i>	++	-	-	-	-	-	-	-	++	+
<i>hisOGDCBHAF</i> - 644	++	-	-	-	-	-	-	-	++	+

Response is indicated according to: ++, growth in 24 hr; +, growth in 48 hr; -, no growth in six-days incubation at 37°.

^a F' factors are *his*⁺ for entire *his* operon except for the specific genes noted in each column.

^b One each *hisD* mutant from the major complementing types was used (*Da*, *Db*, *Dab*).

^c Two separate *hisB* mutants were used in parallel experiments.

^d *hisOGD2233* is representative of five mutants deleted for the same region within the *his* operon.

^e Responds to *Phe* plus *Tyr*.

the mutants to be in fact totally polar, growth in these tests could occur if the F'*his*⁻ mutant reverted on the plate, allowing expression of an intact F'*his*⁺ operon, or if appropriate *Salmonella-E. coli* recombinants were established among the sets of *his*⁻ alleles in each cross. In ruling out these possibilities, we obtained additional auxotrophic markers in several of the *Salmonella* strains to be used as recipient. Clones subsequently scored as positive for complementation were then isolated and their genetic components verified by: (a) mating out the F'*his*⁻ episome; (b) curing the complemented strain of its episome by growing the strain in acridine orange and demonstrating the presence of the original *Salmonella his*⁻ mutant; and (c) testing for absence of conjugation by mating for transfer of markers (*trp*⁺ and *metG*⁺) on either side of the *his* operon.

The possibility that the mutants are only highly polar was also considered. Failure to detect complementation in this case might be theoretically due to intragenic negative complementation. A limited production of the normal *Salmonella* gene polypeptide product, and an adequate production of mutant *E. coli* polypeptides from the corresponding gene could result in nonfunctional enzyme polymers. This is not likely. No evidence for such effects has appeared in *Salmonella hisD*⁻ and *E. coli F'hisD*⁻ mutant complementation tests: a survey involving 150 *S. typhimurium* mutants showed this type of intragenic complementation to be essentially identical with the pattern found in abortive transduction tests involving only *Salmonella*.²⁰ The results in Table 2 are equally negative using episomal *hisD*⁻ mutants of either complementing (*Da* or *Db*) or noncomplementing type (*Dab*). Moreover, the *hisG*, *hisC*, and *hisA* genes have never shown evidence of intragenic complementation of any type.⁶

Failure to express these intact genes cannot be due to the lack of normal translation initiators for these genes. Mutant *hisDC129* is a deletion which has lost a region extending to either side of the *hisD-hisC* boundary. This mutant is

transducible to wild type by *his-3601* and *hisOG203*. Comparable transduction of *hisOGDCB57* may be effected by using mutants deleting the distal portion of the *his* operon. Presence of the normal *hisD* translation initiator in strains with *his-3601* or *hisOG203* may be deduced from the fact that both of these will revert to capacity to grow on histidinol as a source of histidine by events which map in *hisG*.^{21, 22}

We conclude there are two natural initiators internal in the histidine operon which we designate P2 and P3. P2 is in *hisC* or at the *hisC-hisB* boundary, and P3 is in *hisF* or at the *hisF-hisI* boundary (see Fig. 1).

Previously published data from abortive transduction studies of *hisOG203* and *hisOGDCB57* gave different interpretations of the nature of polarity in these mutants.⁸ Detection of intergenic complementation is greatly increased in partial diploids using episomes, relative to the minute colonies formed in abortive transduction. However, the scoring of abortive transductants itself has been facilitated more recently by the use of amino acid supplements.⁶ We have examined strains SC278 and *hisOG203* in reciprocal tests of abortive transductions using three *D*, three *C*, three *B*, one *CB*, two *H*, three *I*, and one each of *F*, *A*, and *E* *his*⁻ mutants; *hisOGDCB57* was examined similarly using the *H*, *A*, *F*, *I*, and *E* mutants. Our data from abortive transduction are all consistent with the patterns seen in episomal complementation.

The deletion mutations involving *hisO* which end in *hisC* or in *hisF*, ie., *hisOGDC63*, *hisOGDC538*, *hisOGDCBHAF644*, do complement for the genes directly following them in the operon order. Although their properties are consistent with the evidence for P2 and P3, lack of polar effects by these tests may arise from the effect of a promoter coupled by each deletion from outside the operon. Existence of such promoter capacity has been suggested by others as a basis for expression of relatively nonpolar *hisOG* deletions.¹ These latter mutants, of which *hisOG1304* is shown in Figure 1 as an example, were obtained from *OG203* by mutation to growth on histidinol as a source of histidine.¹ Of the available deletion mutants ending distal to *hisG*, only *hisOGDCB2226* is interpreted to be in a category analogous to *hisOG1304*.

The degree of expression of distal genes due to the function of P2 or P3 has been considered. From Table 2 this expression in merodiploids is typically sufficient for growth at least within 48 hours. In the absence of the functional pathway, for example in *hisOG203* without any F'*his* factor, P2 appears to be only minimally expressed. Previous assays of extracts of *hisOG203* for the *hisB* enzyme histidinol phosphate phosphatase failed to detect any activity (less than 0.05 wild-type repressed level¹⁵). Our assays of histidinol phosphatase confirm this observation with respect to both *hisOG203* and to SC278. We did find qualitative evidence for the presence of the *hisA* enzyme, isomerase, however. Using crude extracts of *hisOG203* cells, isomerase was also undetected, but reproducible activity was found after partial purification (protamine sulfate and ammonium sulfate fractionations similar to the published procedure¹⁶). Extracts of *hisHA134* were treated identically as a control, and yielded no evidence of isomerase activity. Assays of *hisI* or *hisE* as the result of P3 expression have not been attempted.

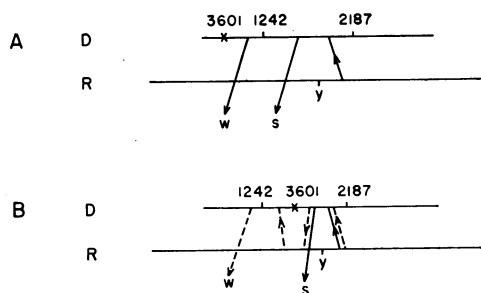


FIG. 2.—Transduction mapping of *his-3601*. Donors (*D*) and recipient (*R*). Dotted lines represent quadruple crossovers. Arrows depict the crossovers for wrinkled (*W*) or smooth (*S*) transductant colony types for alternative positions *A* and *B* of *his-3601*. As recipient, *y* represents *hisG70* or *hisG205*, whose relative mapping positions appear in Fig. 1. Data is presented in Table 3.

Properties of *his-3601*: *His-3601* is a revertable mutant which maps by transduction in the region covered by the deletion mutant *hisOG203*. Further mapping has taken advantage of the property of histidine-constitutive mutants to form wrinkled colonies on plates with high glucose.²³ Mapping crosses are diagrammed in Figure 2. The method assumes a greater frequency of double crossover events than quadruple events in this region. This is supported by recovery of a higher proportion of wrinkled transductants when the more proximal *hisG205* mutant is used as recipient relative to when *hisG70* is used (Table 3). By this method the relative mapping order is *his-3601*, *hisO1242*, *hisG*. Mutant *his-3601* has been more extensively mapped in this position by D. B. Fankhauser and P. E. Hartman using additional *hisO* constitutive mutants.²⁴

TABLE 3. Transduction mapping of mutation *his-3601*.

Strain	Colony Type		Per cent wrinkled
	Smooth (S)	Wrinkled (W)	
<i>hisG70</i>	677	148	18
<i>hisG205</i>	334	129	28

From studies presented elsewhere mutant *his-3601* does not appear to be suppressible.²¹

Induced initiator mutants: A class of point initiator mutants arising in *hisOG203* has been described by Marie St. Pierre.²² These mutants, characterized by *his-1306*, map in *hisG* and allow expression of *hisD* as detected by growth of the strains upon histidinol as a source of histidine. When SC278 was plated upon histidinol revertants were isolated which were shown to be auxotrophic when tested upon minimal medium. These revertants are also due to mutations in *hisG*. They comprise three separate categories as distinguished by their properties after subsequent reversion of the *his-3601* mutation. The functional level of these induced initiators has not been determined.

Discussion. Numerous studies indicate the *his* operon to be a repressible system of the Jacob-Monod type. Although operator-constitutive mutants are known, no mutations unique to the *his* promoter have been described. *His-3601* and extended deletion mutants of the *hisOG203* type may owe their highly polar properties to a number of possibilities involving either transcription or translation. Whether it represents a deficiency in transcription or translation, *his-3601* maps proximal to some known operator constitutive mutants. Thus, at least part of the *his* operator is indicated to lie distal to P1.

The failure of expression of intact genes in *his-3601*, *hisOG203*, and similar deletions involving the proximal region is not due to the lack of potentially functional translation initiators. Goldberger and colleagues have shown two modes of derepression of the *his* operon, sequential and simultaneous.³ Simultaneous derepression is interpreted as due to the attachment of ribosomes at the beginning of all genes in the *his* polycistronic mRNA. Since we have shown the nonfunction of at least two genes proximal to P2 and P3, we interpret P2 and P3 as being transcription initiators.

These initiators have not been detected in repression studies with a functional P1 present. This is not surprising in the case of P2 which appears from enzyme assays to be expressed at only very low levels in the absence of P1. Moreover, expression of P1 may dominate over any secondary promoters; there is suggestive evidence of this in the tryptophan operon.²⁵⁻²⁷ It is possible that the relatively high expression of the internal initiators as seen by growth in complementation tests is related to *in vivo* enzyme stabilization in the presence of the other enzymes in the pathway. It is also conceivable that the product of an additional element proximal to *hisO* accentuates expression of the internal initiators. In our test this product would be provided by the complementing episome.

Fine structure mapping of P2 and P3 has not yet been attempted. Should they map internally in the structural genes, P2 and P3 could represent initiators analogous to certain prototrophic mutations in the tryptophan operon.²⁸ It is possible that they both map at gene boundaries, and may be vestiges of formerly efficient promoters in a preoperon stage of evolutionary development. In this regard, the first internal promoter described, P2 in the *S. typhimarium trp* operon, does map at the boundary between structural genes.²⁷ It is likely that this *trp* P2 is a transcription initiator in view of data obtained with the closely related *E. coli trp* operon.²⁶

The use of nonrecombinant complementation in operon analysis offers a number of possible advantages. Strains with *hisOG203* have a normal *D* translation initiator. Any mutationally derived initiators in *hisD* detectable when *hisOG203/F'hisC*⁻ reverts to grow on minimal medium would thus be transcription initiators. Similarly, partial diploids with *hisOGDCB57* allow the search for transcription initiators arising in *hisH* and *hisA*. Extension of the same methods may detect natural or mutationally derived intragenic translation initiators, or mutationally derived internal transcription terminators. The latter should be detected were they to arise in the genes *G*, *D*, *B*, *H*, or *A* in the *his* operon.

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Since this work was completed, Dr. P. E. Hartman has indicated an additional mutant *his-2321* to be a histidinol nonutilizing strain whose mutation maps under the region of *hisOG203*. It will recombine with two proximal *hisG* mutants (*hisG2101* and *hisG200*) but not with *his-3601*. We find *his-3601* and *his-2321* to be identical as regards their patterns of complementation with episome-borne mutants.

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