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Genetics

Regulation of histidine operon does not require *hisG* enzyme

(*hisG* deletions/repression control/relief of polarity)

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ABSTRACT **Mutations are described which delete all or part of the first structural gene (*hisG*) of the histidine operon of *Salmonella typhimurium*. Physiological regulation of histidine enzymes occurs normally in strains carrying any deletion that has both endpoints within the *hisG* gene. Constitutive high operon expression is observed in strains carrying any *hisG* deletion and an unlinked regulatory mutation, *hisT1504*. These results strongly indicate that the *hisG* protein is not an essential component of the mechanism for regulating expression of the histidine operon.**

The biosynthesis of histidine in *Salmonella typhimurium* requires ten enzymes. The structural genes for these enzymes are located in a cluster of coordinately regulated genes (1, 2) called the histidine operon. Six classes of regulatory mutations have been identified which cause constitutive high level expression of the operon. These are *hisR*, *hisS*, *hisT*, *hisU*, *hisW*, and *hisO*. Characteristics of each of the classes are described in recent review articles (3, 4). None of these classes has been demonstrated to alter any protein that serves directly as a repressor or activator protein. Several lines of evidence have suggested that the *hisG* enzyme ATP phosphoribosyltransferase [*N*-1-(5′-phosphoribosyl) ATP: pyrophosphate phosphoribosyltransferase, EC 2.4.2.17] might be the missing regulatory element (5–11). This protein is the first enzyme in the biosynthetic pathway and is encoded by the first structural gene in the operon.

In the work reported here, mutants lacking all or part of the *hisG* gene have been selected as less polar derivatives of *hisG* frameshift mutations. The general method is similar to that used previously by Jackson and Yanofsky (12). These *hisG* deletions were mapped at high resolution with known point and deletion mutations. Regulation of the *his* operon was then studied in strains containing *hisG* deletions. This paper describes the isolation and mapping of the deletions and the results of studies of their regulatory properties.

MATERIALS AND METHODS

Bacterial Strains. Genotypes and sources of *S. typhimurium* strains are listed in Table 1. The strongly polar point mutations *hisG6608* and *hisG6609* were isolated by selection for temperature resistance of a strain containing the *his01242* constitutive regulatory mutation (13). Mutations *hisG6608* and *hisG6609* were separated from the *his01242* mutation by transduction. Strains containing the numerous *his* mutations used in localizing endpoints of *hisG* deletions (Fig. 2) were obtained from P. E. Hartman and B. N. Ames.

Growth Media. Difco nutrient broth was used as the maximally supplemented liquid medium, with 2 g of agar per 100 ml added for solid medium. The E medium of Vogel and Bonner (14) was used, with 0.2 g of glucose per 100 ml added, as minimal liquid salts medium. Solid minimal salts medium contained 2 g of glucose per 100 ml to permit observation of wrinkled colony morphology (15, 16). Amino acids, when used, were added at a concentration of 50 μg/ml unless otherwise noted. L-Histidinol was added at a concentration of 125 μg/ml.

Transduction Procedures. Growth of phage followed the method of G. Roberts in this lab, modified by the use of enriched nutrient broth (17). To a 1 ml overnight culture of bacteria in nutrient broth was added 5 ml of enriched nutri-

Table 1. Bacterial strains*

Strain	Genotype	Source
TA81	*his01242 hisG6608*	Joyce McCann
TA253	*his*$^+$ *dhuA*$^+$ *hisT1504*	Bruce Ames
TA471	*his*▽*2253 dhuA*$^+$ *hisT1504*	Bruce Ames
TA1690	*his*▽*2253 dhuA1 hisT*$^+$	Larry Kier
TA2327	*his01242 hisG6609*	Joyce McCann
TR3129	*hisO*$^+$ *hisG6608*	Tadahiko Kohno
TR3133	*hisO*$^+$ *hisG6609*	Tadahiko Kohno
		Episome transfer into
TR3308	*hisG6609/F′ hisB2405*	TR3133
TR3309	*hisG6608/F′ hisB2405*	TR3129
		AT-resistant mutant
TR3310	*his*▽*8473/F′ hisB2405*	TR3308
TR3313	*his*▽*8475/F′ hisB2405*	TR3308
TR3314	*his*▽*8476/F′ hisB2405*	TR3309
TR3315	*his*▽*8474/F′ hisB2405*	TR3308
TR3317	*his*▽*8477/F′ hisB2405*	TR3309
		Transduction into
TR3335	*his*▽*8473 dhuA1*	TA1690
TR3336	*his*▽*8473 hisT1504*	TA471
TR3339	*his*▽*8475 dhuA1*	TA1690
TR3340	*his*▽*8475 his T1504*	TA471
TR3343	*his*▽*8476 dhuA1*	TA1690
TR3344	*his*▽*8476 hisT1504*	TA471
TR3347	*his*▽*8474 dhuA1*	TA1690
TR3348	*his*▽*8474 hisT1504*	TA471
TR3351	*his*▽*8477 dhuA1*	TA1690
TR3352	*his*▽*8477 hisT1504*	TA471
TR3355	*hisC483 dhuA1*	TA1690
TR3356	*hisC483 hisT1504*	TA471
TR3359	*hisC537 dhuA1*	TA1690
TR3360	*hisC537 hisT1504*	TA471
TR3502	*hisG6609 hisT1504*	TA471
TR3504	*hisG6608 hisT1504*	TA471
TR3505	*his*$^+$	LT-2

* All strains are derived from *S. typhimurium* LT2. Mutation *his-2253* deletes *hisO, G, D, C, B,* and part of the *hisH* gene. The *dhuA1* mutation facilitated strain construction and is not pertinent to studies of regulation of *his* biosynthetic enzymes.

Abbreviations: *HT* phage, high-frequency transducing phage; AT, 3-amino-1,2,4-triazole.

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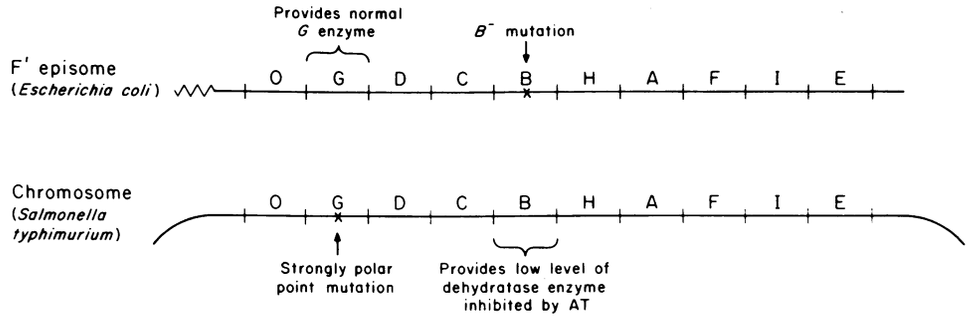

FIG. 1. Genetic structure of strains used in isolation of *hisG* deletions.

ent broth containing 10^8 plaque-forming units/ml of the appropriate phage. The culture was incubated overnight, cells and debris removed by low-speed centrifugation, and the supernatant was decanted into a fresh tube and sterilized by shaking with a few drops of chloroform. The chloroformed phage stock was allowed to stand at room temperature overnight, and then stored at 4°. A titer of 10^{10}–10^{11} plaque-forming units/ml was usually obtained by this method with the phage types listed below.

Phage types used for strain construction were P22 (*int-4*) or P22 (*HT int*$^-$), a derivative of P22 (*HT*) (ref. 18; obtained from B. A. D. Stocker) into which an *int* mutation was introduced by G. Roberts in this lab. Phage types used for transduction mapping were P22 (*HT*), or P22 (*HT int*$^-$). Prior to use, *HT* phage were grown for at least one cycle on a host that carried mutation *his-3050* which deletes the entire *his* operon. This assured that no transductants arose in mapping crosses due to carry-over of *his*$^+$ material from the previous host.

Initial screening for *hisG* deletions was done by spreading 0.2 ml of an overnight nutrient broth culture of recipient cells on minimal glucose + 0.005 mM histidine plate, and delivering 25 small drops of donor phage suspensions on the plate. The final verification of *hisG* deletion endpoints was obtained by spreading 0.1 ml of the *hisG* deletion mutant as recipient plus 0.1 ml of the appropriate donor phage lysate (10^{10}–10^{11} plaque-forming units/ml) together on a series of plates containing minimal glucose + 0.005 mM histidine. Crosses finally scored as negative gave no recombinants in tests that would have elicited greater than 100,000 recombinants with a wild type donor. Strains built by transduction were single colony isolated selectively and were shown to be free of phage (19).

Enzyme Assays. Toluenized whole cell assays have been described for the *hisB* enzyme histidinolphosphate phosphatase (L-histidinol-phosphate phosphohydrolase, EC 3.1.3.15) (20), and the *hisD* enzyme histidinol dehydrogenase (L-histidinol: NAD$^+$ oxidoreductase, EC 1.1.1.23) (21). Growth media for repressing and derepressing conditions contained E salts + glucose (0.2 g/100 ml) and either 0.1 mM histidine or 1.0 mM histidinol, respectively. Cells were grown to mid-log phase in 50 or 100 ml cultures on a rotary shaker at 37°, collected by centrifugation, washed three times, and then resuspended in the buffer appropriate for the assay to be done. Optical density (650 nm) of the washed cell suspension was used to calculate enzyme specific activities.

RESULTS

Rationale

Much of the *in vivo* evidence that has been used to implicate the product of the first structural gene (*hisG*) of the *his* operon of *S. typhimurium* as a predominant element in regulating expression of this operon has been indirect. To clarify this situation we have isolated a series of deletion mutants which lack extensive regions of the *hisG* gene. With these deletion mutants it is possible to assess the *in vivo* regulatory function of the *hisG* enzyme in a straight-forward manner.

Isolation of *hisG* deletion mutants

Mutants lacking large regions of the *hisG* gene were selected as less polar derivatives of the strongly polar frameshift mutants *hisG6608* or *hisG6609*. The genetic structure of parental strains (TR3308 and TR3309) used in the selection is shown in Fig. 1. These strains carry, in addition to one of the polar *hisG* mutations, the F'*hisB2405* episome of *Escherichia coli* origin which does not recombine detectably with the chromosomal *his* region presumably because of the lack of base sequence homology (22). Although the parental strains are phenotypically His$^+$, they contain reduced levels of *hisB* enzyme because of the episomal *hisB* mutation, and the polar effect of the *hisG* mutations on chromosomal *hisB* expression. As a consequence of the reduction in *hisB* enzyme these strains are abnormally sensitive to growth inhibition by 3-amino-1,2,4-triazole (AT), a compound that inhibits *hisB* enzyme activity (23). Among several mechanisms by which AT-sensitivity can be overcome is deletion of the *hisG* polar mutation. The presence of an intact *hisG* gene on the episome enables such deletion mutants to remain phenotypically His$^+$.

The selection was carried out by spreading 0.1 ml portions of overnight cultures of one of the parental strains (inoculated from individual colonies to assure independent isolates) on minimal medium plates containing glucose (2 g/100 ml), AT (15 mM), adenine (0.5 mM), and thiamine (0.05 mM). (Adenine relieves a block in purine biosynthesis produced by AT, and thiamine relieves the adenine sensitivity of *S. typhimurium* strains derepressed for the *his* operon.) AT-resistant clones appearing after 2 days' incubation at 37° were picked, purified, and phage lysates were prepared for preliminary identification of *hisG* deletions. AT-resistant strains whose phage lysates failed to elicit recombinants with several recipients containing different *hisG* point mutations were saved as potential *hisG* deletion mutants. Approximately 10% of the mutants surviving the selection proved to contain deletions of the general type desired. Each *hisG* deletion was transduced into a haploid recipient carrying deletion *his-63* selecting for growth on histidinol. The transductant clones, which by necessity inherited the *hisG* deletion, were used in transductional mapping crosses as described under *Materials and Methods*.

A map of the *hisG* gene showing endpoints of five representative deletions isolated and characterized by these methods is given in Fig. 2. The set of mutations used in mapping

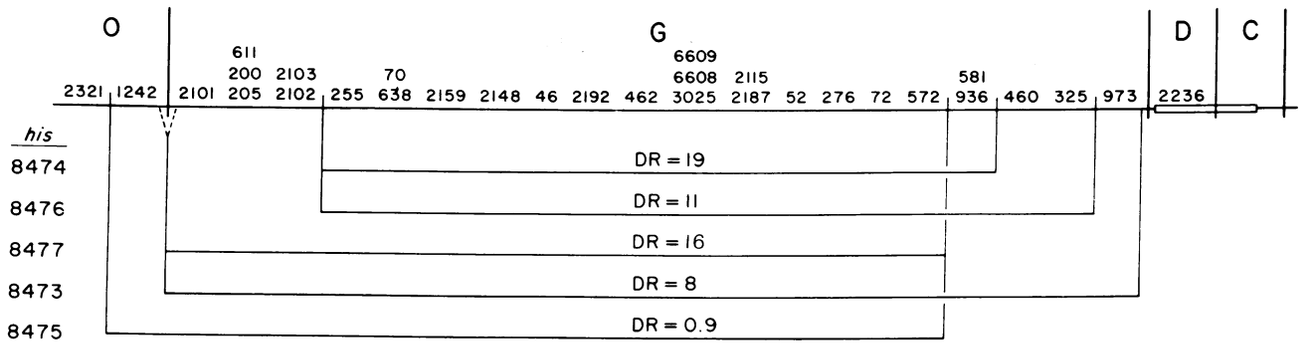

FIG. 2. Map positions of *hisG* deletion mutations. This map is based on phage P22-mediated transductional crosses. Derepression ratios (DR) for strains containing deletion mutations *his-8473–his-8477*, taken from Table 2, are based on assay of the *hisB* enzyme. Deletion mutation *his-8475* removes a part of the *hisO* control region and elicits high constitutive levels of histidine enzymes (DR = 0.9).

includes the most promoter-proximal and most promoter-distal point mutations known in *hisG*. The order of *hisG* point mutations has been revised somewhat based on data obtained in this study.

Regulation of the *his* operon in strains containing *hisG* deletions

Data in Tables 2 and 3 indicate that *hisG* enzyme is unnecessary for repression or derepression of the *his* operon. This conclusion is derived from analysis of physiological regulation (Table 2), and genetic derepression as a consequence of the *hisT1504* (24) regulatory mutation (Table 3), in strains containing the *hisG* deletions shown in Fig. 2.

Strains containing *hisG* deletions that do not extend into the *hisO* control region (Fig. 2) maintain repressed levels of *his* enzymes when grown in minimal media containing excess histidine, and derepress normally when histidine is limited by growth on the histidine intermediate, histidinol (Table 2). With strains containing such internal *hisG* deletions (*his-8473*, *-8474*, *-8476*, and *-8477*), derepression ratios are in the range 11–16 and 8–19 for the *hisD* and *hisB* enzymes, respectively. These values compare with those of 8–9 for *hisD* enzyme and 13–15 for *hisB* enzyme, obtained with the *hisG*$^+$ control strains (*hisC483* and *hisC537*). Strain LT2, used to standardize the repressed level of *his* enzymes (≡1.0), does not contain a *his* mutation and thus does not derepress when grown in the presence of histidinol. Deletion *his-8473* results in reduced levels of *hisD* enzyme activity possibly because of loss of a short segment of the *hisD* gene. Deletion *his-8475* elicits high constitutive levels of *hisD* and *hisB* enzymes. Altered regulation caused by *his-8475* is entirely consistent with a defect in the adjacent *hisO* control region, since this deletion fails to recombine with the constitutive regulatory mutation *hisO1242* and therefore extends through *hisG* into the *hisO* region (Fig. 2).

The *hisG* enzyme is not necessary to mediate genetic derepression caused by the *hisT* regulatory mutation (Table 3). Strains containing an internal *hisG* deletion and the regula-

Table 2. Physiological regulation of the histidine operon in *hisG* deletion mutants

Strain	Relevant genotype	Growth* medium	Doubling time (min)	*hisD* enzyme: Relative specific activity	*hisD* enzyme: Derepression ratio	*hisB* enzyme: Relative specific activity	*hisB* enzyme: Derepression ratio
TR3505	LT2(*his*$^+$)	his	65	≡1.00	—	≡1.00	—
		hol	65	1.70	1.7	1.49	1.5
TR3355	*hisC483*	his	63	1.20	—	1.21	—
		hol	120	10.0	8.3	15.7	13
TR3359	*hisC537*	his	65	1.30	—	1.21	—
		hol	120	11.0	8.5	17.6	15
TR3335	*his*▽*8473*	his	58	0.13	—	0.71	—
		hol	76	2.10	16	5.76	8.1
TR3347	*his*▽*8474*	his	55	1.00	—	0.72	—
		hol	75	14.0	14	13.6	19
TR3343	*his*▽*8476*	his	60	0.90	—	0.92	—
		hol	73	10.0	11	10.5	11
TR3351	*his*▽*8477*	his	65	0.60	—	0.70	—
		hol	75	7.10	12	10.9	16
TR3339	*his*▽*8475*	his	58	8.00	—	10.9	—
		hol	75	11.0	1.4	9.81	0.9
TR3133	*hisG6609*	his	65	≤0.02	—	0.07	—
		hol	75	0.60	≥30	0.76	11
TR3129	*hisG6608*	his	63	≤0.02	—	0.15	—
		hol	76	0.60	≥30	1.04	6.9

* Cells were grown for maximal repression on medium containing 0.1 mM histidine (his). For derepression, cells were grown on medium containing the growth-limiting histidine intermediate histidinol (hol) at a concentration of 1.0 mM.

Table 3. Derepression of *hisG* deletion mutants by the *hisT1504* mutation*

Strain	Relevant genotype	Doubling time (min)	*hisD* enzyme		*hisB* enzyme	
			Relative specific activity	Derepression ratio	Relative specific activity	Derepression ratio
TR3505	LT2 (*his*$^+$)	48	≡1.00	—	≡1.00	—
TA253	*his*$^+$ *hisT1504*	60	19.8	20	12.3	12
TR3355	*hisC483*	55	0.92	—	1.18	—
TR3356	*hisC483 hisT1504*	60	13.1	14	11.4	9.7
TR3359	*hisC537*	54	1.04	—	1.01	—
TR3360	*hisC537 hisT1504*	60	14.6	14	11.7	12
TR3335	*his*∇*8473*	50	0.14	—	0.79	—
TR3336	*his*∇*8473 hisT1504*	60	1.34	9.6	4.82	6.1
TR3347	*his*∇*8474*	42	1.12	—	0.72	—
TR3348	*his*∇*8474 hisT1504*	55	16.0	14	7.62	11
TR3343	*his*∇*8476*	53	0.88	—	1.22	—
TR3344	*his*∇*8476 hisT1504*	60	7.61	8.6	10.1	8.3
TR3351	*his*∇*8477*	52	0.49	—	0.71	—
TR3352	*his*∇*8477 hisT1504*	60	7.78	16	9.54	13
TR3339	*his*∇*8475*	52	6.46	—	5.75	—
TR3340	*his*∇*8475 hisT1504*	58	6.45	1.0	9.23	1.6
TR3133	*hisG6609*	63	≤0.02	—	0.24	—
TR3502	*hisG6609 hisT1504*	65	0.34	≥16	0.63	2.6
TR3129	*hisG6608*	65	≤0.02	—	0.22	—
TR3504	*hisG6608 hisT1504*	65	0.40	≥20	0.59	2.7

* All strains were grown in minimal medium containing 0.1 mM L-histidine.

tory mutation *hisT1504* exhibit derepression ratios comparable to those of *hisG*$^+$ *hisT1504* strains. Again, deletion *his-8473* results in reduced activity of *hisD* enzyme, and deletion *his-8475*, which extends into the *hisO* region, elicits constitutively high *his* enzyme levels regardless of the presence or absence of the *hisT* mutation.

Results are also presented in Tables 2 and 3 for strains containing the polar mutations (*hisG6608* and *hisG6609*) used for selection of *hisG* deletions. These data demonstrate the strong polarity effects of these mutations, as well as the ability of strains carrying chain-terminating *hisG* mutations to derepress both physiologically (Table 2) and in a *hisT1504* background (Table 3). These effects are most clearly observed by measurement of *hisD* enzyme activity. Polar effects on *hisB* expression are less extreme due to the existence of a low level constitutive promoter between the *hisC* and *hisB* genes (21).

In addition to the deletion mutations described in this paper, 19 other deletions with endpoints within the *hisG* gene have been analyzed. Strains containing any of these mutations exhibit normal control of the *his* operon by criteria similar to those used above. By a slightly different isolation procedure, we have also obtained strains containing deletions which extend through the *hisG* gene into the *hisD* and *hisC* genes. These strains exhibit normal regulation as well, indicating that no essential regulatory element is encoded in the distal end of the *hisG* gene, or between the *hisG* and *hisD* genes.

DISCUSSION

Although physiological (5–7) and biochemical (8–10) data have accumulated suggesting involvement of the *hisG* gene product in regulation of the *his* operon of *S. typhimurium*, evidence presented in this paper clearly demonstrates that this protein cannot be an obligatory repressor or activator in the regulatory process. Physiological regulation of the *his* operon in strains carrying extensive deletions of the *hisG* gene occurs in a manner essentially identical to that observed in *hisG*$^+$ strains. The finding that the *hisT1504* regulatory mutation elicits expected constitutive derepression of *his* enzymes in strains containing *hisG* deletions further implies that the *hisG* product is not necessary to mediate effects of the well-established co-regulator of *his* operon expression—His-tRNA^{His} (3). Mutations in *hisT* lead to constitutively derepressed synthesis of *his* enzymes by inactivating an enzyme that converts uridine to pseudouridine (Ψ) in the anticodon loop of tRNA^{His} (25). The Ψ modification is necessary for normal regulatory function of tRNA^{His}, as well as other tRNA species (26).

We think it highly unlikely that all the *hisG* deletions we have characterized would leave intact a promoter proximal portion of the *hisG* gene that codes for a protein fragment essential in the regulation. Several *hisG* deletions (Fig. 2) fail to recombine with *hisG2101*, the *hisG* point mutation most proximal to the adjacent *hisO* regulatory region. Since *hisG2101* itself recombines very infrequently with nearby mutations in the *hisO* region, this implies that *hisG* deletions can end extremely near the *hisO-hisG* border without causing regulatory alterations.

If *hisG* enzyme does participate in regulating expression of the *his* operon its function must be a dispensable one. For example, if two independent regulatory mechanisms exist, only one of which involves *hisG* enzyme, then the deletions described here would leave the second mechanism intact. Artz and Broach (27) have recently described an "activator-attenuator" model of *his* operon regulation which can accommodate an accessory function of *hisG* enzyme. Such an accessory function is not inconsistent with our studies, and the specific mechanism suggested (27) can account for *in vitro* biochemical evidence that has implicated *hisG* enzyme in the regulation.

However, it is difficult to reconcile our evidence obtained with *hisG* deletion mutants and *all* of the data that have

been used to implicate this protein in the regulation. One example of such an anomaly is the report that some function of the *hisG* protein is necessary to mediate repression by the histidine analog 1,2,4-triazole-3-alanine (6, 7). We have examined this phenomenon with several strains containing different *hisG* deletions (unpublished experiments); in each case the *his* operon was repressible by the analog, as well as by histidine.

We believe that our results clearly indicate that *hisG* enzyme is not essential for regulation of the *his* operon in *S. typhimurium*. Similar evidence has ruled out mandatory participation of the first enzymes of the tryptophan (12, 28) and isoleucine (29) pathways in regulation of the *E. coli trp* and *ilv* operons, respectively.

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