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Histidyl-Transfer Ribonucleic Acid Synthetase in Positive Control of the Histidine Operon in *Salmonella typhimurium*[1]

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Histidyl-transfer ribonucleic acid (tRNA) synthetase (HRS), coded by the *hisS* gene, appears to play two roles in regulation of the histidine operon of *Salmonella typhimurium*: (i) in synthesis of a critical effector molecule, histidyl-tRNA, and (ii) a more direct effect elicited by the presence of the enzyme protein itself. The specific activity of HRS was elevated either by mutations in the *strB* locus or in *hisS*$^+$ merodiploids of *Escherichia coli/S. typhimurium* and *S. abony/S. typhimurium*. In each case, an increase in HRS was accompanied by an increase in histidine operon expression, indicating that HRS may be involved in positive control of the histidine operon. It is unlikely that HRS leads to increased histidine operon expression merely by acting as a "sponge" for charged tRNA. Rather, HRS appears to influence operon expression by interaction with some effector molecule other than charged tRNA or by a direct interaction with the histidine operator-promoter region. The functional level of histidine operon expression has no effect on HRS specific activity.

Histidyl-transfer ribonucleic acid (tRNA) synthetase (HRS) in *Salmonella typhimurium* is specified by gene *hisS*, a gene unlinked by P22 transduction to the histidine operon (12, 17). HRS affects regulation of the *his* operon because it participates in synthesis of histidyl-tRNA, and the amount of charged tRNA is critical in regulation (7). Here we present evidence that HRS plays a second role in regulation, namely, that HRS exerts an action as a positive effector of operon expression. A preliminary report of some of this work has been presented (J. H. Wyche et al., Abstr. Annu. Meet. Amer. Soc. Microbiol. 1972, G75, p. 43).

MATERIALS AND METHODS

Bacterial strains. The bacterial strains used in this study are listed in Table 1. Genetic symbols are defined in Sanderson (14).

Transduction tests. Transduction experiments were performed with the use of P22 mutant *int-4* (16) by direct plating together of phage and bacteria (B. Ely, R. M. Weppelman, H. C. Massey, Jr., and P. E. Hartman, Genetics, submitted for publication). Media, growth of bacteria and phage for use in genetic experiments, and F' matings are described elsewhere (15; Ely et al., manuscript in preparation).

Isolation of strB mutations. *strB* mutants are resistant to low levels of streptomycin and, in addition, some are auxotrophic for thiamine and nicotinic acid (5). For isolation of *strB* mutants, the strain was grown overnight in broth containing 600 μg of 2-aminopurine nitrate/ml, and 0.1 ml was plated on nutrient agar plates containing 50 μg of streptomycin sulfate/ml. After overnight incubation at 37 C, the plates were replica plated onto minimal glucose plates and minimal glucose plates supplemented with 1 μg each of thiamine and nicotinic acid/ml. All plates also contained other supplements as necessary, depending on the growth requirements of the parental strains. Colonies that grew on the plates containing thiamine and nicotinic acid but did not grow in the absence of these supplements were considered to be *strB* auxotrophs, and those with low reversion frequencies were chosen for further use. More recent isolations of *strB* mutations have shown that mutagenesis is unnecessary because auxotrophic *strB* mutants occur spontaneously with a frequency of approximately 10^{-6}. An additional attribute of *strB* mutants is their relative resistance to other aminoglycoside antibiotics such as neomycin (19).

Isolation of FS403. An F' carrying the locus for the histidyl-tRNA synthetase gene (*hisS*) was isolated by crossing a multiply marked auxotrophic Hfr strain with a *recA strB* recipient and examining appropriately prototrophic clones for further transfer of the *strB*$^+$ allele. *S. abony* strain SW1403 (HfrH2 *met-469 aro-164 strA H1^b H2^{enx}*) was chosen as the donor since

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TABLE 1. *Bacterial strains used in the study*

Strain	Genotype	Reference or source
S. typhimurium LT-2		
SB847	*recA101 hisBH22*	(20)
SB2051	*hisO3150 fla-2055*	(B. Ely, manuscript in preparation)
SB2319	*recA101 hisBH22 strB651*	2-AP[a] mutagenesis of SB847
SB2320	*recA101 hisBH22 strB652*	2-AP[a] mutagenesis of SB847
SB2341	*recA101 hisBH22 strB651*/FS403	Mating SW1403 with SB2319
SB2474	*recA1 leu-1115 guaA1 strA*	P. E. Hartman stock collection
SB2475	*recA1 leu-1115 guaA1 strA*/FS403	Contact of SB2341 with SB2474
SB2476	*recA101 hisBH22 strB652*/FS403	Contact of SB2475 with SB2320
SB2477	*hisS6331 hisC6330*	J. H. Wyche (Ph.D. thesis, Johns Hopkins, 1971)
SB2510	*hisS1587 hisC6330 strB660*	J. H. Wyche (Ph.D. thesis, Johns Hopkins, 1971)
SB2511	*hisS2280 hisC6330 strB661*	J. H. Wyche (Ph.D. thesis, Johns Hopkins, 1971)
SB2512	*hisS6301 hisC6330 strB662*	J. H. Wyche (Ph.D. thesis, Johns Hopkins, 1971
SB2513	*hisS1520 hisC6330 strB663*	J. H. Wyche (Ph.D. thesis, Johns Hopkins, 1971
SB2514	*hisS3173 hisC6330 strB664*	J. H. Wyche (Ph.D. thesis, Johns Hopkins, 1971)
SB2617	*hisO1242 fla-2055*	B. Ely (manuscript in preparation)
SB2618	*hisO1812 fla-2055*	B. Ely (manuscript in preparation)
SB2625	*his⁺ fla-2055*	B. Ely (manuscript in preparation)
SB2790	*hisG46 strB668 fla-2055*	2-AP[a] mutagenesis of SB3095
SB2800	*hisO2321*	P. E. Hartman stock collection
SB2994	*hisS1587*	B. Ely (Ph.D. thesis, Johns Hopkins, 1973)
SB2995	*hisS2280*	B. Ely (Ph.D. thesis, 1973)
SB2996	*hisS6301*	B. Ely (Ph.D. thesis, 1973)
SB2997	*hisS1520*	B. Ely (Ph.D. thesis, 1973)
SB3036	*hisO1812 hisS6301*	B. Ely (Ph.D. thesis, 1973)
SB3048	*his⁺ strB668*	SB2790 transduced to *his⁺*
SB3067	*hisS1587 hisC6330 strB660*/F'142	Contact of SB3072 with SB2510
SB3068	*hisS2280 hisC6330 strB661*/F'142	Contact of SB3072 with SB2511
SB3069	*hisS6301 hisC6330 strB662*/F'142	Contact of SB3072 with SB2512
SB3070	*hisS1520 hisC6330 strB663*/F'142	Contact of SB3072 with SB2513
SB3071	*hisS3173 hisC6330 strB664*/F'142	Contact of SB3072 with SB2514
SB3072	*recA1 leu-1115 guaA1 strA*/F'142	Contact of SB3073 with SB2474
SB3073	*his-3217 guaA1*/F'142	Simultaneous contact of KLF142/KL253 (CGSC4279) and SB5404 (fer⁻ for reduced restriction) and SB3192
SB3074	*his-515 met-522 fla-1001*/FS400 *hisD2981*	Contact of TA1904 with SB2626
SB3095	*hisG46 fla-2055*	P. E. Hartman stock collection
SB3097	*strB57 fla-2056*	P. E. Hartman stock collection
SB3104	*his⁺ strB⁺ fla-2055*	SB2625 phage x SB3048
SB3105	*hisG46 strB670 fla-2055*	2-AP[a] mutagenesis of SB3095
SB3106	*hisG46 strB671 fla-2055*	2-AP[a] mutagenesis of SB3095
SB3123	*hisG46 strB670 fla-2055*/F'142	Contact of SB3072 with SB3105
SB3178	*tyrA3*/F'142	Contact of SB3072 with SB3190
SB3190	*tyrA3*	B. Ely (Ph.D. thesis, 1973)
SB3192	*his-3217 guaA1*	P. E. Hartman stock collection
SB3209	*hisO2321 strB676*/F'142	Contact of SB3072 with SB3212
SB3210	*hisO1812 hisS6301 strB675*/F'142	Contact of SB3072 with SB3211
SB3211	*hisO1812 hisS6301 strB675*	Spontaneous in SB3036
SB3212	*hisO2321 strB676*	Spontaneous in SB2800
SB3214	*hisG46 strB671 fla-2055*/F'142	Contact of SB3072 with SB3106
SB3217	*strB57 fla-2056*/F'142	Contact of SB3072 with SB3097
SB3218	*hisO1812 strB668 fla-2055*	SB2618 phage x SB2790

TABLE 1—*Continued*

Strain	Genotype	Reference or source
SB3219	*hisO1812 strB668 fla-2055*/F'142	Contact of SB3072 with SB3218
SB8042	*hisO1242 hisF3042*	P. E. Hartman stock collection
SB8050	*his-3050* (deletion of entire *his* operon) *ara-9*	P. E. Hartman stock collection
S. typhimurium LT-7		
SB3107	*hisD10 strB672 gal-24*	Spontaneous in SB5010
SB3108	*hisD10 strB673 gal-24*	Spontaneous in SB5010
SB3109	*hisD10 strB673 gal-24*	Spontaneous in SB5010
SB3124	*hisD10 strB672 gal-24*/F'142	Contact of SB3072 with SB3107
SB3215	*hisD10 strB673 gal-24*/F'142	Contact of SB3072 with SB3108
SB3216	*hisD10 strB674 gal-24*/F'142	Contact of SB3072 with SB3109
SB5404	*hisI404 proC51 strA667 fer*	P. E. Hartman stock collection
S. abony		
SW1403	HfrH2 *met-469 aro-164 strA* $H1^b$ $H2^{enx}$	(15)
E. coli		
KLF142/K1253	(CGSC4279) *tyrA2 pyrD34 thi-1 his-68 trp-45 recA1·mtl-2 xyl-7 malA1 galK35 str-118* λ^r λ^-/*F'142*	Barbara J. Bachmann, *E. coli* Genetic Stock Center, Yale Univ. School of Medicine

[a] 2-AP = 2-aminopurine.

integration of F has occurred close to *hisS* in this strain (Fig. 1; 14, 15). Because *strB* is closely linked to *hisS*, an appropriate recipient strain was constructed by selecting a *strB* mutation in strain SB847 (*recA101 hisBH22*) as described above to give strain SB2319 (*recA101 hisBH22 strB651*).

Samples of 0.1 ml of donor and recipient bacteria were inoculated into individual tubes each containing 3 ml of nutrient broth and incubated for 2 h without aeration at 37 C. A 1-ml portion of each culture was filtered onto a sterile, moistened membrane filter (Millipore Corp., 0.45 μm, type HAWP). The filter was placed on a prewarmed, freshly poured nutrient soft agar plate (0.75%). After 30 min of incubation at 37 C, the filter was removed, placed in 1 ml of minimal glucose medium, and agitated in a Vortex mixer for 2 min. Samples of 0.1 ml were spread on minimal glucose medium plus 20 μg of L-histidine/ml and incubated at 37 C for 3 days. Approximately 125 *strB*$^+$ *met*$^+$ *aro*$^+$ recombinants appeared per plate. Forty recombinants were picked and streaked onto minimal glucose plus histidine plates. After growth, they were replica plated onto a minimal glucose plate and onto a minimal glucose plate prespread with a culture of *strB57* (SB3097). One streak which failed to grow on minimal medium but did transfer *strB*$^+$ to *strB57* was selected as a presumed merozygote strain containing an F' *strB*$^+$ (FS403). The phenotype of this strain (SB2341 = *strB651 recA101 hisBH22*/FS403) is StrB$^+$ RecA$^-$ His$^-$, and StrB$^-$ clones are segregated at high frequency.

Growth of bacteria for enzyme assays. Single colonies were inoculated into 1 to 2 ml of Difco nutrient broth and grown overnight at 37 C. The next day, 0.05 ml of the broth culture was added to 16 $\times$ 150 mm tubes containing 1 to 1.5 ml of minimal glucose plus a concentration of appropriate supplements 10-fold higher than the final concentration ultimately desired. After overnight growth at 37 C without aeration, the cultures were diluted to 10 ml with minimal glucose and aerated at 37 C in a bubbler tube. Cells resumed exponential growth without a lag period. Growth was monitored periodically with a Klett-Summerson colorimeter at 660 nm. When the turbidity of a culture reached 95 to 120 Klett units (mid log phase), the culture was placed on ice until all cultures were ready for assay. When larger cultures were desired, 0.05 or 0.1 ml of an overnight broth culture was inoculated into 5 ml of minimal glucose in a 25 by 200 mm tube, incubated at 37 C overnight without aeration, and diluted to 40 ml the next morning.

Preparation of sonically disrupted extracts. Forty-milliliter cultures were centrifuged at 12,000 $\times$ *g* for 5 min in a Sorvall refrigerated centrifuge. The pellets were resuspended and washed in 10 ml of 0.1 M tris(hydroxymethyl)aminomethane (Tris) buffer, pH 7.5, and sedimented again. To each pellet, 2 ml of 0.1 M Tris, pH 7.5, were added, and the resulting suspension was treated for 1 min at 1.6 A on an MSE ultrasonic power unit. The extracts were centrifuged at 40,000 $\times$ *g* for 20 min to remove cellular debris. A 1.0-ml portion of each supernatant fluid was applied to a small Sephadex G-25 column (1.2 by 9 cm) previously equilibrated with 0.1 M Tris buffer, pH 7.5. The peak protein fraction was collected in a volume of 1.0 ml and assayed for enzyme activity and protein.

Preparation of toluene-treated cells. Toluene-treated cell suspensions were prepared as described elsewhere (1).

Enzyme assays. HRS activity was measured by the histidine-dependent pyrophosphate exchange reaction in sonically treated extracts as described by Cebula (manuscript in preparation). L-Histidinol phosphate phosphatase (EC 3.1.3.15), the *hisB* en-

zyme, was measured on toluene-treated cells as described by Ely (manuscript in preparation). Except where noted, all values for *hisB* activity are the average of two or more determinations with each determination assayed in duplicate, and standard deviations of the assay values are less than 20%.

The assay of toluene-treated cells for histidinol dehydrogenase (EC 1.1.1.23), the *hisD* enzyme, is a modification of the procedure of Martin et al. (9) as diagrammed in Table 2. The dye mix is photosensitive at high pH, so all manipulations involving it were carried out in dim light, and the rack of assay tubes was wrapped in foil. Reagents were added in the order listed, and the reaction was started by the addition of histidinol. After incubation at 37 C in the dark, the reaction was stopped by the addition of 0.5 N HCl. The color was stabilized by the lowered pH and the absorbance was measured at 520 nm. Activity was calculated according to the formula $10[2(A - B) + (C - D)]$, where A, B, C, and D stand for the absorbance of tubes A, B, C, and D respectively (Table 2). Units were defined as OD_{520} per ml of toluene-treated cells per hour. The activity was divided by the absorbance at 650 nm to correct for protein concentration. Wild type has a specific activity of 0.5 units when grown in minimal glucose medium supplemented with 0.1 mM histidine.

Protein assays. Protein was determined by the method of Lowry et al. (8), employing bovine serum albumin as the standard.

RESULTS

Genetic content of FS403. A strain of *S. typhimurium* carrying an F′ (FS403) derived from *S. abony* was prepared as described in Materials and Methods. The genetic composition of the F′ in this strain (SB2341 = *strB651 recA101 hisBH22*/FS403) was discerned as follows. Samples of a culture of strain SB2341 were spotted on plates of minimal medium previously spread with strains containing mutations in the *strB* region of the *S. typhimurium* chromosome (Fig. 1). Prototrophic recombinants were formed with strains having the following individual markers: *purG310*, *glyA1*, *strB57*, *guaA1*, *purI305*, *cysA20*, and *ptsHI2309*. Thus, FS403 was presumed to carry the wild-type alleles of these loci. Similarly, *hisS*+, the structural gene for HRS, was demonstrated to be carried by FS403. The FS403-carrying strain, SB2341, was cross-streaked against strain SB2477 (*hisS6331 hisC6330*) on minimal glucose plates and incubated at 25 C. The *hisS6331*

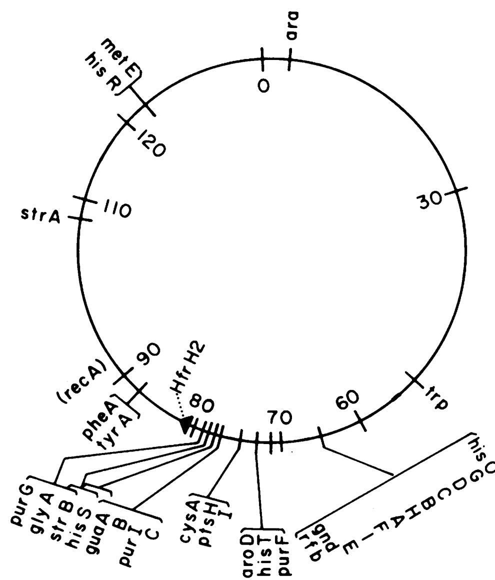

FIG. 1. *A partial genetic map of S. typhimurium (adapted after reference 14).*

TABLE 2. *Histidinol dehydrogenase (hisD enzyme) activity.*

Additions	Tube A	Tube B	Tube C	Tube D	Zero
0.2 M TEA buffer, pH 8.6	0.30 ml	0.30 ml	0.30 ml	0.30 ml	0.30 ml
Toluene-treated cells	0.075 ml	0.075 ml	0.15 ml	0.15 ml	
0.1 M TEA buffer, pH 7.5	0.075 ml	0.075 ml			0.15 ml
Dye mixture[a]	0.30 ml	0.30 ml	0.30 ml	0.30 ml	0.30 ml
Histidinol[b]	30 µliters		30 µliters		
0.5 N HCl[c]	0.15 ml	0.15 ml	0.15 ml	0.15 ml	0.15 ml

[a] Dye mixture is made fresh in a brown bottle immediately prior to use by mixing: 5 parts of 3.2 mg of 2-*p*-iodophenyl-3-*p*-nitrophenyl-5-phenyl-tetrazolium chloride per ml dissolved by heating to boiling in a foil-covered flask and then stored and refrigerated in the dark; 1 part 0.4 mg/ml phenazine methosulfate (stored refrigerated in the dark); 1 part of 0.2% gelatin (stored refrigerated), 1 part of 20 mg of NAD (β-nicotinamide adenine dinucleotide per ml, stored frozen).

[b] A 100 mM amount of L-histidinol-monoHCl (ethanol free) is adjusted to pH 8,0 with TEA base. After histidinol addition, shake to mix and incubate in the dark for 20 min at 37 C. (Time can be increased when low activities are expected.)

[c] Measure absorbance at 520 nm.

mutation engenders an HRS with an elevated K_m for histidine and leads to an absolute growth requirement for histidine (17), whereas the auxotrophic effects of the temperature-sensitive mutation, *hisC6330*, are relieved at 25 C. Prototrophic recombinants in the cross-streak test at 25 C indicated the presence of the *hisS*$^+$ gene on FS403 as well as the dominance of *hisS*$^+$ to *hisS6331* with regard to prototrophy. The above nine loci carried by FS403 are contiguous on the *S. typhimurium* chromosome (Fig. 1; 14). In contrast, no prototrophic recombinants were formed in cross-streak tests with strains containing other markers known to be nearby on the *S. typhimurium* chromosome: *pheA10*, *aroD5*, and *purF145*. Thus, we conclude that prototrophs were formed by transfer of FS403 and not by aberrant chromosomal mobilization in the *rec*$^-$ donor strain. FS403 contains a region of the *Salmonella* chromosome with termini between *purG* and *pheA* (79 to 87 min on the map in Fig. 1) and between *pts* and *purF* (74 min). Also, we conclude that *S. typhimurium* and *S. abony* share a high degree of genetic homology in this chromosomal region.

Effects of FS403 on enzyme levels. Data in the top two lines of Table 3 show that HRS activity is elevated in a strain carrying FS403

TABLE 3. *HRS and his B enzyme levels of strains diploid for hisS*[a]

Strain	Relevant genotype	HRS sp act[b] (U/mg)	*hisB* enzyme sp act[c] (U/OD_{650})
SB2474	*hisS*$^+$ *recA1 guaA1*	1.6	4.7
SB2475	*hisS*$^+$ *recA1 guaA1*/FS403 *hisS*$^+$ *guaA*$^+$	4.6	8.3
SB3072	*hisS*$^+$ *recA1 guaA1*/F'142 *hisS*$^+$ *guaA*$^+$ *tyrA*$^+$	4.4	6.5
SB3192	*hisS*$^+$ *guaA1*	1.4	1.3
SB3073	*hisS*$^+$ *guaA1*/F'142 *hisS*$^+$ *guaA*$^+$ *tyrA*$^+$	2.9	2.2
SB3190	*hisS*$^+$ *tyrA3*		1.3
SB3178	*hisS*$^+$ *tyrA3*/F142 *hisS*$^+$ *guaA*$^+$ *tyrA*$^+$		3.0

[a] Cells were grown in minimal glucose medium supplemented with 0.1 mM L-histidine (repressed conditions) and other supplements as necessary.

[b] HRS specific activity was measured by using the standard histidine-stimulated inorganic pyrophosphate-adenosine 5'-triphosphate exchange assay on extracts prepared by sonication (Materials and Methods).

[c] *HisB* enzyme-specific activities are the average of two or more determinations with each determination performed in duplicate.

(SB2475) when compared with its immediate parent lacking FS403 (SB2474). Cordaro and Roseman (2) have demonstrated a similar increase in the enzymes dictated by the *ptsH* and *ptsI* genes when FS403 is present. These increases are typical of increased gene dosage due to diploidy for the structural genes in question. Unexpected, however, is the observation that expression of the histidine operon also is elevated in strain SB2475 (last column of Table 3) because the *his* operon is not carried by the FS403 episome.

Effects of an E. coli F'. An F' derived from *Escherichia coli* K12 (F'142) carries a region of the *E. coli* chromosome similar in genetic content to that of FS403. The bacterial genes on the *E. coli* episome are sufficiently non-homologous with those of the *S. typhimurium* recipient so that rather stable merodiploids are formed even in *rec*$^+$ bacteria. The lower portion of Table 3 shows that F'142 elicits increased *hisS* and *his* operon expression in two different *S. typhimurium* strains. Therefore, the elevation of *his* operon expression is not due to properties unique to a particular recipient strain, F', or species combination.

Effects of strB mutations. Mutations in the *strB* locus lead to low-level (50 to 100 μg/ml) streptomycin resistance and to auxotrophy for thiamine and nicotinic acid (5). Mutations in *strB* are pleiotropic in that they also increase HRS activity about 30% (compare SB3095 and SB3105 in Table 4). Expression of the histidine operon also is enhanced in *strB* strains (last column of Table 4). At the bottom of Table 4 are data obtained on strain SB2790 and two successively isolated transductional clones (SB3048 and SB3104); these data indicate that the elevated levels of HRS and *hisB* enzymes are the result of the *strB* mutation rather than some secondary mutation.

Dominance of strB$^+$. Increased *his* operon expression in *strB* strains is substantially relieved after introduction of F'142 which carries the *strB*$^+$ gene (Table 4). The decrease in *hisB* enzyme in the merodiploids indicates that effects of *strB* mutations on histidine enzyme levels are recessive to *strB*$^+$. Although lowered, the *his* operon enzyme levels do not return to normal in the merodiploids. Because these strains also are diploid for the *hisS*$^+$ gene and thus would contain elevated HRS activity due to gene dosage (cf. Table 3), the remaining elevation in *his* operon expression could result indirectly from the presence of excess HRS (see below).

Further experiments suggest that one *strB*$^+$ allele may suffice for normal HRS expression

TABLE 4. *Effect of strB mutations on hisB enzyme and hisS enzyme levels*[a]

Strain	Relevant genotype	HRS sp act[b] (U/mg)	*hisB* enzyme sp act[c] (U/OD_{650})
SB2625	*his*$^+$	1.4 ± 0.1	1.4
SB3095	*hisG46*	1.5 ± 0.1	1.6
SB3105	*hisG46 strB670*	2.0 ± 0.1	3.4
SB3123	*hisG46 strB670*/F'142 *strB*$^+$		3.3
SB3106	*hisG46 strB671*		3.8
SB3214	*hisG46 strB671*/F'142 *strB*$^+$		2.2
SB3107	*hisD10 strB672*		3.7
SB3124	*hisD10 strB672*/F'142 *strB*$^+$		3.2
SB3108	*hisD10 strB673*		4.8
SB3215	*hisD10 strB673*/F'142 *strB*$^+$		3.6
SB3109	*hisD10 strB674*		5.1
SB3216	*hisD10 strB674*/F'142 *strB*$^+$		4.1
SB3097	*strB57*		3.6
SB3217	*strB57*/F'142 *strB*$^+$		2.7
SB2790	*hisG46 strB668*	1.9 ± 0.1	3.8
SB3048	*his*$^+$ *strB668*		4.2
SB3104	*his*$^+$ *strB*$^+$	1.4 ± 0.1	1.6

[a] Cells were grown in minimal glucose medium supplemented with 0.1 mM histidine. Nicotinic acid and thiamine each were added at a concentration of 2 μg/ml where required.

[b] The values for HRS activity represent the average of three or more determinations with each determination done in quadruplicate.

[c] The values for *hisB* enzyme activity represent single determinations assayed in duplicate.

and that *strB*$^+$ is dominant to *strB* with respect to HRS expression. The top two lines of Table 5 show that a *S. typhimurium* mutant strain, SB847, contains increased *his* operon levels. These increased levels are due to a mutation of unknown location. In spite of the unusual nature of this strain, it is evident that both HRS and *his* operon expression are enhanced when *strB* mutations are introduced into strain SB847 (= SB2320 and SB2319). The addition of an episome containing *strB*$^+$ *hisS*$^+$ elevates HRS activity because of increased gene dosage while *his* operon activity again is lowered (Table 6). The increases in HRS levels of the *strB*/*strB*$^+$ merodiploids are comparable with those found for *strB*$^+$/*strB*$^+$ merodiploids (Table 3), again suggesting that *strB* is recessive to *strB*$^+$.

Effects of growth rate. The pleiotropic *strB* mutations lengthen the bacterial generation time (19). Therefore, strain SB3095 (*hisG46*) was grown on citrate as sole carbon source where it exhibits the same generation time (70 min) as do *strB* strains when grown on glucose. HRS and *hisB*$^+$ enzyme levels are unchanged in citrate-grown strain SB3095 from those found in glucose-grown cultures (data not shown). Thus, a change in growth rate, per se, is not responsible for increased HRS and *his* operon enzyme levels in *strB* strains. Similarly, the presence or absence from the medium of nicotinic acid and thiamine, required for growth by *strB* strains,

TABLE 5. *HRS and hisD enzyme levels in strains with elevated expression of hisS*[a]

Strain	Relevant genotype	HRS sp act (U/mg)	*hisD* enzyme sp act[b] (U/OD_{650})
SB2625	*hisS*$^+$ *his*$^+$	1.4	0.5
SB847	*hisS*$^+$ *hisBH22 recA101*	1.6[c]	1.2[c]
SB2320	*hisS*$^+$ *hisBH22 recA101 strB652*	2.2	2.1
SB2476	*hisS*$^+$ *hisBH22 recA101 strB652*/FS403 *hisS*$^+$ *strB*$^+$	3.1	1.4
SB2319	*hisS*$^+$ *hisBH22 recA101 strB651*	2.0	2.4
SB2341	*hisS*$^+$ *hisBH22 recA101 strB651*/FS403 *hisS*$^+$ *strB*$^+$	2.9	1.7

[a] Cells were grown in minimal glucose medium supplemented with 0.1 mM histidine. Nicotinic acid and thiamine each were added at a concentration of 2 μg/ml where required.

[b] *hisD* enzyme specific activities are the average of two or more determinations each performed in duplicate.

[c] SB847 and its derivatives contain a mutation of unknown location that leads to an approximately twofold increase in *his* operon enzyme levels above the content found in other LT-2 sublines.

TABLE 6. *HRS levels in strains altered for the expression of the his operon*[a]

Strain	Relevant genotype	HRS sp act (U/mg)	*hisB* enzyme sp act (U/OD_{650})
SB2625	*his*$^+$ *hisS*$^+$	1.4	1.4
SB8050	*his-3050 hisS*$^+$	1.3	<0.1
SB2051	*hisO3150 hisS*$^+$	1.5	4.6
SB8042	*hisO1242 hisF3042 hisS*$^+$	1.4	36[b]

[a] Cells were grown in minimal glucose medium supplemented with 0.1 mM histidine.

[b] *hisB* enzyme assay was done on SB2617 (*hisO1242 hisF*$^+$ *hisS*$^+$).

has no effect on the growth rate of strain SB3095 or on the rate of *his* operon expression.

Independence of HRS expression. *strB* mutations simultaneously affect both *hisS* and *his* operon expression. These two effects could be either independent or coupled. Therefore, a *strB* mutation was selected in strain SB2800 (*hisO2321*), a mutant with negligible expression of the primary *his* promoter (Ely, manuscript in preparation). The HRS level in the resulting *strB* strain was found to be elevated and comparable with the HRS levels of *strB* strains with normal *his* operon regulation (data not shown). Therefore, *his* operon expression is not necessary for the elevated HRS levels of *strB* mutants.

A further experiment clearly demonstrates the independence of HRS levels from products of the *his* operon (Table 6). The HRS activity is the same in a wild-type strain and in a strain carrying a deletion of the entire *his* operon (*his-3050*), as well as in two operator constitutive strains (*hisO3150* and *hisO1242*) with *hisB* enzyme levels of 3 and 25 times that of wild type, respectively.

HRS mediates his operon expression. Data described above show that expression of *hisS* and the *his* operon behave in a parallel fashion. The evidence also suggests that this coordination is not effected through products of the *his* operon. Data in Table 7 show that four out of five *hisS* mutants tested as merodiploids carrying F'142 contain nearly wild-type levels of *his* operon enzyme (1.6 to 2.3 units of *hisB* enzyme/OD_{650}); among the five *hisS* mutants only *hisS2280* reveals a clearly higher *his* operon expression typical of most $hisS^+/hisS^+$ diploids (cf. data in Tables 3 and 4). The data of Table 7 suggest that most *hisS* mutations lower the effect of diploidy on histidine operon expression and thus implicate HRS directly in the control of *his* operon expression.

Data obtained with a constitutive mutant, *hisO1812*, further implicates HRS in *his* operon control and again suggests that the increased his operon expression of *strB* strains may be mediated through wild-type HRS (Table 8). *his* operon expression is increased by a *strB* mutation or by diploidy for the $hisS^+$ gene (top three lines of Table 8), but no increases due to *strB* or *hisS* diploidy are found in the presence of the *hisS6301* mutation (bottom three lines of Table 8). It thus seems likely that the mechanism for the elevated *his* operon expression of *strB* mutants is the same as the mechanism for elevated *his* operon expression of strains diploid for $hisS^+$. Namely, *strB* mutations serve to increase HRS activity, and the increased HRS activity then secondarily serves to elevate *his* operon expression.

TABLE 7. *hisB enzyme levels in strains containing mutations in hisS*[a]

Strain	Relevant genotype	*hisB* enzyme sp act[b]	
		+his (U/OD_{650})	−his (U/OD_{650})
SB2994	*hisS1587*	11.3 (60)[c]	18 (66)
SB2510	*hisS1587 hisC6330*[d] *strB660*	4.8 (120)	
SB3067	*hisS1587 hisC6330 strB660*/F'142[e]	2.3 (65)	
SB2995	*hisS2280*	11.9 (53)	33 (77)
SB2511	*hisS2280 hisC6330 strB661*	6.2 (120)	
SB3068	*hisS2280 hisC6330 strB661*/F'142	3.9 (60)	
SB2996	*hisS6301*	11.0 (60)	30 (57)
SB2512	*hisS6301 hisC6330 strB662*	4.1 (85)	
SB3069	*hisS6301 hisC6330 strB662*/F'142	2.0 (57)	
SB2997	*hisS1520*	1.7 (65)	30 (100)
SB2513	*hisS1520 hisC6330 strB663*	5.1 (120)	
SB3070	*hisS1520 hisC6330 strB663*/F'142	1.6 (54)	
SB2514	*hisS3173 hisC6330 strB664*	4.0 (88)	
SB3071	*hisS3173 hisC6330 strB664*/F'142	2.1 (50)	

[a] The K_ms for L-histidine, ATP and $tRNA_{his}$ are, respectively (reference 3): wild type (25 μM, 140 μM, 110 nM); *hisS1587* (5 μM, 20 μM, 815 nM); *hisS2280* (22 μM, 625 μM, 105 nM); and *hisS1520* (905 μM, 1990 μM, 76 nM).

[b] Cells were grown in minimal glucose medium. A 0.1 mM amount of histidine was added to the growth medium where indicated. Nicotinic acid and thiamine were added at a concentration of 2 μg/ml where needed. Values for *hisB* enzyme are the average of two or more determinations each performed in duplicate.

[c] Numbers in parentheses indicate doubling time in minutes.

[d] Mutation *hisC6330* is a temperature sensitive, nonpolar mutation with no effect on *hisB* enzyme levels when excess histidine is present in the growth medium.

[e] F'142 contains $hisS^+$ $strB^+$.

DISCUSSION

Elevation of HRS levels. The specific activity of HRS in *S. typhimurium* is elevated in two situations: (i) in strains that carry mutations in the *strB* locus (Tables 4, 5, and 8) and (ii) in $hisS^+$ merodiploids (Tables 3 and 8). Because histidyl-tRNA is involved in regulation of HRS synthesis (10, 11), one possibility is that histidyl-tRNA is limiting in these strains and HRS production is consequently derepressed. Such an indirect mechanism is particularly plausible in the case of merodiploids where *E. coli* and *S. abony* $hisS^+$ genes are present in a *S.*

TABLE 8. *hisB enzyme levels in strains containing hisS6301*[a]

Strain	Relevant genotype	*hisB* enzyme sp act[b] (U/OD_{650})
SB2618	*hisO1812 hisS*$^+$	14
SB3218	*hisO1812 hisS*$^+$ *strB668*	41
SB3219	*hisO1812 hisS*$^+$ *strB668*/ F'142 *strB*$^+$	49
SB3036	*hisO1812 hisS6301*	21
SB3211	*hisO1812 hisS6301 strB675*	20
SB3210	*hisO1812 hisS6301 strB675*/ F'142 *strB*$^+$	16

[a] *hisS6301* is a mutation in the *hisS* gene which leads to an altered HRS protein and to increased *hisB* enzyme (Table 7).

[b] Cells were grown in minimal glucose medium supplemented with 0.1 mM L-histidine. SB3218 and SB3211 cultures also contained 2 μg of thiamine and nicotinic acid/ml.

typhimurium genetic background because HRS is a dimeric enzyme composed of two subunits (T. A. Cebula, manuscript in preparation; F. DeLorenzo and A. Schechter, manuscript in preparation). By random subunit association, much of the HRS in the merodiploids could be composed of mixed subunits and could be catalytically less active than the respective homomeric enzymes. This might lead to limiting HRS and thence to derepression of HRS production. We do not think this is the case because the amount of HRS activity determined on cell extracts of the diploids is about that expected based on gene dosage (Table 3) and found for two other enzymes coded for by one of the same F-primes (2). Furthermore, *strB* mutations increase both *his* enzyme and HRS levels (Tables 5 and 8), whereas they decrease enzyme levels of the *his* operon in mutants with defective HRS where histidyl-tRNA is presumed to be limiting (Table 7). Because histidyl-tRNA also is a vital component in repression of the *his* operon (7), it would appear that the slowed growth properties of *strB* strains enhance the opportunity for charging of $tRNA_{his}$ rather than decreasing charging in strains with *hisS* mutations (Table 7). Our tentative conclusions are that the level of functional HRS is truly increased in both of the circumstances mentioned above and that charged histidyl-tRNA is not a limiting factor in *strB* strains or in *hisS*$^+$ merodiploids.

Increased his operon expression. When HRS levels are elevated we find that *his* operon expression also is enhanced. We propose that elevated HRS secondarily stimulates enzyme production by the *his* operon and that this stimulation is potentiated in the presence of *strB* mutations. So far only a twofold stimulation of *his* operon expression with elevated HRS has been observed, whereas histidine enzyme levels can vary over a 20-fold range. Therefore, other regulatory mechanisms could be superimposed on the HRS effect. For example, a different conformation of HRS also could serve as a repressor. Alternatively, greater HRS stimulation may take place but, because HRS is an essential cell component always present, we may only detect a portion of the total stimulatory effect that actually occurs. In any event, although HRS affects the *his* operon, the converse is not true; that is, *his* operon function has no effect on HRS specific activity (Table 6 and text).

The mechanism of HRS stimulation of *his* operon expression remains to be elucidated in detail. In fact, it is possible that HRS per se is not the regulatory molecule. Because there is rapid turnover of HRS when higher than normal levels are present (10, 18), it is possible that regulation is not affected by "native" HRS itself but, rather, by a modified product.

One way HRS could act would be to bind the presumed co-repressor, histidyl-tRNA, effectively withdrawing it from a role in repression. For example, in the model of HRS turnover just mentioned, defective HRS might "trap" tRNA but not aminoacylate it. Even an increase in wild-type, "native" HRS might effectively trap tRNA. The internal pool of $tRNA_{his}$ is approximately 2,300 nM and of HRS is roughly 2,200 nM (3). Because the K_m of $tRNA_{his}$ is 110 nM (4) and the K_D for $tRNA_{his}$ and histidyl-$tRNA_{his}$ are both approximately 40 nM (J. A. Lewis, Ph.D. thesis, Univ. of California, Berkeley, 1972), it seems reasonable to assume that excess HRS could prevent charged $tRNA_{his}$ from playing a role in repression. However, mere binding of histidyl-$tRNA_{his}$ seems an inadequate explanation for the role of HRS on *his* operon expression. First of all, some strains with mutations in the histidine operator region are subject to repression but fail to elevate *his* operon expression in response to *strB* mutations (Ely, manuscript in preparation). In a similar vein, operator mutations such as *hisO1812* respond effectively to *strB* mutations (Table 8), but are insensitive to mutations in regulator genes *hisR* and *hisT* (Ely, manuscript in preparation) that strongly influence the amount of histidyl-$tRNA_{his}$ effective in repression (7).

A second way HRS could influence *his* operon expression would be through protein-protein

interactions with some other effector molecule. Evidence for an interaction of the tryptophanyl-tRNA synthetase and the tryptophan repressor has been obtained by Ito (6).

Finally, a third mechanism for the stimulatory role of HRS on *his* operon expression could reside in interactions directly at the histidine operator region, namely, HRS could serve as an "activator" in positive control of the *his* operon. This possibility is discussed separately in conjunction with a detailed presentation of the properties of histidine operator mutants (Ely, manuscript in preparation).

The strB locus. Mutations in the *strB* locus are pleiotropic: (i) they give rise to low-level resistance to a number of aminoglycoside antibiotics (5, 19); (ii) they engender requirements for thiamine and nicotinic acid (5); (iii) they lengthen the generation time (19, Table 7); and (iv) they increase HRS levels and, as mentioned above, potentiate the effects of increased HRS on *his* operon expression (Tables 4, 5, and 8).

We have found that all effects of strB mutations are recessive to $strB^+$. Finally, we carried out preliminary experiments in which amino acid-stimulated inorganic-pyrophosphate-adenosine 5'-triphosphate exchange was measured in crude extracts under conditions optimal for histidine stimulated exchange in order to determine if the level of HRS was specifically affected or if there is a more general effect on aminoacyl-tRNA synthetases. Our preliminary data indicate that of the amino acids that display significant activity (isoleucine, leucine, phenylalanine, lysine, methionine, cystine, valine, and tryptophan), only lysyl-tRNA synthetase activity is significantly affected by the presence of a *strB* mutation. In *strB* strains, the lysine-stimulated exchange is about 30% lower than that found in $strB^+$ strains.

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