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Regulation of Histidyl-Transfer Ribonucleic Acid Synthetase Formation in a Histidyl-Transfer Ribonucleic Acid Synthetase Mutant of *Salmonella typhimurium*

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Control of formation of the histidyl-transfer ribonucleic acid (tRNA) synthetase with an increased K_m for histidine was studied in a *hisS* mutant of *Salmonella typhimurium*. Histidine restriction of both the *hisS* and *hisS*$^+$ strains resulted in a derepression of synthesis of histidyl-tRNA synthetase. When grown in a concentration less than the K_m (100 μg/ml) of L-histidine, the *hisS* mutant maintained a higher level of histidyl-tRNA synthetase than the *hisS*$^+$ strain. Addition of excess amounts of L-histidine to the growth medium of the *hisS* mutant culture grown with 100 μg of L-histidine per ml resulted in a repression of histidyl-tRNA synthetase formation to equal that of the *hisS*$^+$ strain grown in 100 μg of L-histidine per ml. These data confirm previous findings that histidine tRNA is involved in the repression of synthesis of histidyl-tRNA synthetase.

The results of several studies on the regulation of aminoacyl-transfer ribonucleic acid (tRNA) synthetase formation in *Escherichia coli* and *Salmonella typhimurium* have suggested the involvement of the free amino acid and its cognate tRNA in the repression process (5, 10). Initially, Williams and Neidhardt (10) reported that histidyl-tRNA synthetase formation responded to a manipulation of the histidine supply to the cells. Addition of excess histidine to a histidine-restricted culture caused a decrease in the rate of synthesis of histidyl-tRNA synthetase. Conversely, removal of excess histidine from the growth medium resulted in an elevated rate of histidyl-tRNA synthetase formation. These results suggested the importance of histidine in the control of the histidyl-tRNA synthetase formation.

Studies involving histidine regulatory mutants of *S. typhimurium* have suggested the importance of histidine tRNA ($tRNA^{His}$) in controlling the rate of formation of histidine biosynthetic enzymes (7). Silbert et al. (8) have successfully demonstrated that the synthesis of the histidine biosynthetic enzymes was nonrepressible in a *hisR* mutant lacking half of wild-type $tRNA^{His}$. Consistent with these findings, McGinnis and Williams reported nonrepressibility of histidyl-tRNA synthetase formation in a *hisR* mutant during histidine-unrestricted growth (5). In view of the results of Williams and Neidhardt (10) and McGinnis and Williams (5), the present study was undertaken to test directly the notion that the histidyl-tRNA synthetase plays a role in regulating the rate of synthetase formation. The data in this paper suggest that this idea is correct and that histidyl-tRNA is the end-product effector for that regulation.

MATERIALS AND METHODS

Organisms. Three strains of *S. typhimurium* were used in this study. SP1 (*ara9 metE338 ilvC401 strA149*) is *hisS*$^+$ and auxotrophic for histidine. Strain SB2449 (*hisS1520, hisC6330*) is a *hisS* mutant. Strain SB2450 (*hisC6630*) possesses the same *hisC* mutation as strain SB2449 and is *hisS*$^+$. Strains SB2449 and SB2450 were obtained from P. Hartman.

Media and methods of cultivation. The growth medium used in this study was the basal salts solution of Fraenkel and Neidhardt (3), supplemented with 0.4% glucose and 0.2% ammonium sulfate as carbon and nitrogen sources. For unrestricted growth of these organisms, a concentration of 100 μg of all required amino acids (L-isomer forms) per ml was used. To achieve limitation of the growth rate by

histidine, 0.5 to 2.0 μg of glycyl-DL-histidine per ml was used. This low concentration of histidine was used to achieve a severe histidine restriction and at the same time permit a slow, but continuous, supply of histidine to the cells. Additional amino acid supplements are specified in individual experiments. Growth was measured by an increase in optical density at 420 nm with a 1-cm light path in a Hitachi-Perkin-Elmer, model 111 spectrophotometer.

Preparation of cell extracts. As described by Chrispeels et al. (2), cells were subjected to sonic treatment with a Branson sonic oscillator. Protein content of extracts was determined by using the method of Lowry et al. (4).

Preparation of tRNA. Cells were grown in unrestricted medium, and 40-ml samples were taken at each one-half mass doubling for 2.5 generations of growth. Samples were immediately added to tubes containing 10 ml of 100% trichloroacetic acid. After centrifugation at 4 C, the pellet was suspended in 0.05 M sodium acetate buffer (*p*H 5.5) containing 0.06 M KCl and 0.01 M $MgCl_2$. The cells were subjected to sonic treatment with a Branson sonic oscillator, as described by Williams and Freundlich (9). Phenol extraction, ethanol precipitation, and column fractionation were as described elsehwere (9). After column fractionation, the 1 M LiCl fractions were pooled.

In vivo charging procedure. The tRNA preparations were subjected to periodate oxidation, and the isolated tRNA was deacylated of amino acids by incubation for 1 hr in 0.1 M tris(hydroxymethyl)aminomethane (Tris)-hydrochloride (*p*H 8.9; 37 C) as described earlier (9). The RNA content was determined by absorbance at 260 nm by using 24 absorbancy units to equal 1 mg of RNA per ml. The tRNA samples were used in the standard attachment assay system, with ^{14}C-histidine as the amino acid substrate and with excess enzyme, as described elsewhere (2).

Histidinol phosphate phosphatase assay. Except for several modifications, the assay was performed by the method of Ames et al. (1). The reaction mixture, which was incubated at 37 C for 30 min, consisted of 0.04 ml of crude extract, 0.22 ml of 0.1 M triethanolamine-hydrochloride (*p*H 7.5) and 0.02 ml of histidinol phosphate to give a final volume of 0.28 ml. Ascorbic acid-plus-molybdate reagent was added to terminate the reaction. At a wavelength of 820 nm, the tubes were read against an enzyme and buffer blank without histidinol phosphate.

Histidyl-tRNA synthetase assay. Using the procedures of Chrispeels et al. (2), we determined histidyl-tRNA synthetase activity by the attachment assay system by using ^{14}C-histidine and excess substrates, with the exception that incubation was at 37 C for 5 min. One unit of activity was defined as that which formed 1 μmole of product per hr. Specific activity was expressed as units per milligram of protein.

Source of chemicals. Uniformly labeled L-[^{14}C]-histidine was obtained from New England Nuclear Corporation (Boston, Mass). *E. coli* K-12 tRNA was purchased from General Biochemical Corp. (Chagrin Falls, Ohio).

L-Histidinol and L-histidinol phosphate were purchased from Sigma Chemical Co. (St. Louis, Mo.), and glycyl-DL-histidine from Mann Research Laboratory (New York).

RESULTS

The *hisS* mutant, SB2449, employed in this study has been described elsewhere (6). Roth and Ames (6) reported that the histidyl-tRNA synthetase of this mutant possessed a K_m for histidine of 80×10^{-4} M compared to 1.5×10^{-4} for this enzyme from the wild-type strain, as measured by the pyrophosphate exchange assay (activation step). For the attachment assay (transfer step), the enzyme of the mutant strain exhibited a K_m for histidine 12-fold greater than that of the enzyme from the wild-type strain (i.e., 0.5×10^{-4} M). In our laboratory, the wild-type enzyme has a K_m for histidine of 0.66×10^{-4} M, and the mutant enzyme has a K_m for histidine of approximately 10.6×10^{-4} M, as measured by the attachment assay. Our determinations were made by using a crude extract which did not permit very accurate measurements of the affinity for histidine; yet the results are consistent with those of Roth and Ames (6) and adequate for the experiments reported in this paper. In addition, as shown in Fig. 1, the mutant possesses an unaltered affinity for histidine RNA.

Level of histidinol phosphate phosphatase in hisS$^+$ and hisS strains of S. typhimurium during histidine-unrestricted growth. Figure 2 shows the differential rate of synthesis of his-

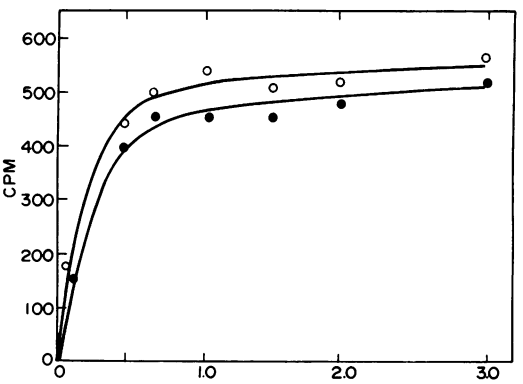

FIG. 1. *Transfer ribonucleic acid saturation curve for histidyl-tRNA synthetase of the hisS mutant and hisS$^+$ strains. As described in Materials and Methods, increasing concentrations of tRNA were used in the standard attachment assay system. The closed circles represent hisS$^+$, and the open circles represent the hisS mutant. The data are plotted counts per min per 2 μg of protein as a function of increasing tRNA concentration.*

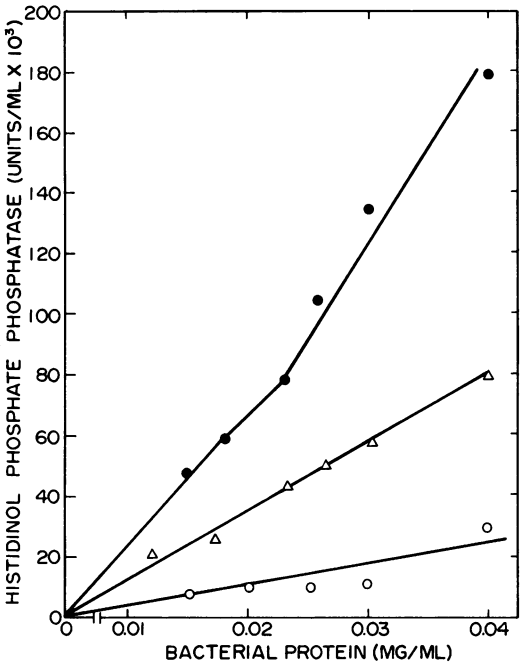

FIG. 2. *Effect of histidine-unrestricted growth on the differential rate of formation of histidinol phosphate phosphatase in the hisS⁺ (SP1) and the hisS mutant (SB2449) strains. The cells were initially grown in histidine-unrestricted medium, harvested, washed twice, and transferred to one flask containing 200 μg and to another containing 100 μg, respectively, of L-histidine per ml in the case of the mutant. The hisS⁺ strain, SP1, was transferred to medium containing only 100 μg of L-histidine per ml. The differential rate of synthesis was determined for both the hisS⁺ strain, SP1, during growth in 100 μg of L-histidine per ml (O——O), and for the hisS strain, SB2449, in 100 μg of L-histidine per ml (●——●) and 200 μg of L-histidine per ml (Δ——Δ). Results are plotted as enzyme units per milliliter of culture as a function of total protein per ml of culture.*

tidinol phosphate phosphatase in both the mutant and wild-type strains during histidine-unrestricted growth conditions. In addition, the *hisS* mutant was also grown in the presence of 200 μg of L-histidine per ml, which is in excess of its K_m for histidine (Fig. 2). There was an apparent derepression of histidinol phosphate phosphatase synthesis in the mutant culture grown with 100 μg of L-histidine per ml, which is less than its K_m for histidine. In the presence of 200 μg/ml, however, there was a twofold reduction in the rate of synthesis of histidinol phosphate phosphatase in the *hisS* mutant. As expected, the wild-type strain exhibited a low level of histidinol phosphate phosphatase during histidine-unrestricted growth.

Differential rate of histidyl-tRNA synthetase formation in hisS⁺ and hisS mutant during histidine-unrestricted growth. From the same enzyme preparations used to measure the level of histidinol phosphate phosphatase, the relative differential rate of histidyl-tRNA synthetase formation was determined for both the *hisS* mutant and *hisS* strains (Fig. 3). Similarly, a supplement of 100 μg of L-histidine per ml allowed the enzyme of the mutant to be made at a rate three times that of the wild-type strain for several mass doublings (Fig. 3). Consistent with the result shown in Fig. 2 for histidinol phosphate phosphatase formation, the relative differential rate of formation of histidyl-tRNA synthetase in the mutant equaled that of the wild type when grown with sufficient L-histidine to overcome the K_m difference (200 μg/ml) (Fig. 3).

It should be noted that strain SB2449 possesses a temperature-sensitive *hisC* product, which would have become inactivated during growth at 37 C, and the expression of the histidine operon would be altered. Thus, the same

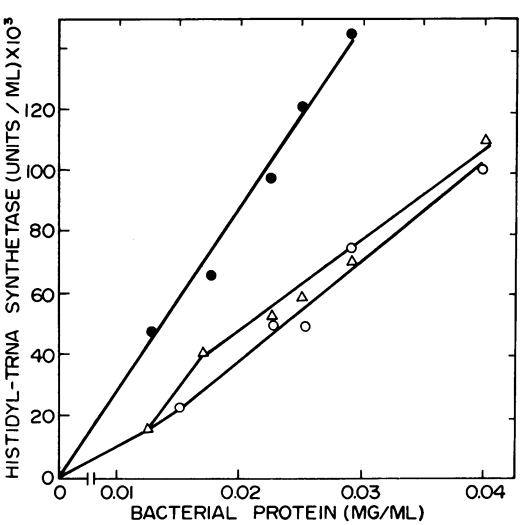

FIG. 3. *Effect of histidine-unrestricted growth on the differential rate of formation of histidyl-tRNA synthetase in the hisS⁺ (SP1) and hisS mutant (SB2449). From the same extracts used to determined the level of histidinol phosphate phosphatase in both the hisS mutant and hisS strains, the relative differential rate of histidyl-tRNA synthetase formation was determined for both hisS⁺ strains grown in 100 μg/ml (O——O), hisS mutant grown in 100 μg/ml (●——●), and hisS mutant grown in 200 μg of L-histidine per ml (Δ——Δ). Results are plotted as enzyme units per milliliter of culture as a function of total protein per milliliter of culture.*

experiment was performed with strain SB2450 which carries the same temperature-sensitive *hisC* mutation, but is *hisS*$^+$. The results shown in Fig. 4 indicate that the rate of histidyl-tRNA synthetase formation in this strain grown in minimal medium supplemented with 100 or 200 μg of L-histidine per ml is essentially equal to the rate of synthesis observed for the other *hisS*$^+$ strain (SB2449). Therefore, the results were not influenced by the temperature-sensitive character of the *hisC* mutation of strain SB2449 (Fig. 3 and 4).

Histidyl-tRNA synthetase formation during restricted growth of mutant and wild-type strains. After growth of both strains in histidine-unrestricted media, the cells were washed several times and suspended in histidine-restricted minimal media. Figure 5 is a plot of the differential rate of synthesis of histidyl-tRNA synthetase in the mutant grown in the presence of 2 μg of glycyl-DL-histidine per ml and the wild type grown in 1 μg of glycyl-DL-histidine per ml. The data suggest that in the mutant the relative differential rate of histidyl-tRNA synthetase formation during the derepression phase was about three times greater than the rate of synthesis of this enzyme in the wild-type strain.

In vivo charging of tRNA^{His} in hisS$^+$ and hisS mutant strains. To correlate further

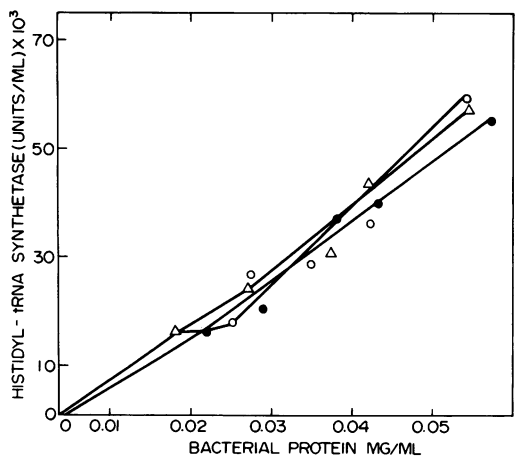

FIG. 4. *Effect of histidine-unrestricted growth on the differential rate of formation of histidyl-tRNA synthetase in strains SB2450 and SP1. The experimental details are as described in Fig. 3. The differential rate of histidyl-tRNA synthetase formation was determined for SB2449 grown in 100 μg of L-histidine per ml (●——●), SB2450 grown in 100 μg (○——○) and 200 μg (△——△) of L-histidine per ml. Results are plotted as enzyme units per milliliter of culture as a function of total protein per milliliter of culture.*

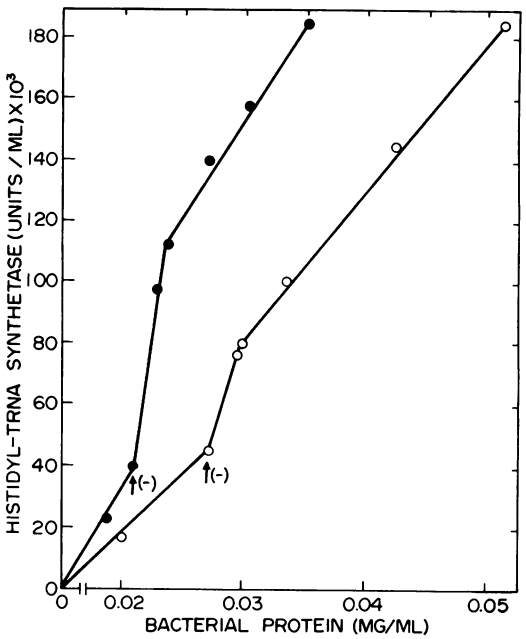

FIG. 5. *Effect of histidine-restricted growth on the differential rate of formation of histidyl-tRNA synthetase in the hisS$^+$ and hisS mutant strains. The cells were initially grown in histidine-unrestricted medium, harvested, washed twice with minimal medium, and transferred to histidine-restricted medium (1 μg of glycyl-D L-histidine per ml for the hisS, and 2 μg of glycyl-D L-histidine per ml, for the mutant. The activity was determined for histidyl-tRNA synthetase in both the hisS$^+$ (○——○) and hisS mutant (●——●) strains. The data are plotted as described in Fig. 3. Histidine restriction occurred at the time indicated by the arrow.*

hisS activity and control of synthesis of this enzyme, we measured the in vivo charging of histidine tRNA. The mutant was grown for several generations in 100 and 200 μg, respectively, of L-histidine per ml, and samples were taken at one-half mass doublings. The wild type was grown under the same conditions in 100 μg of L-histidine per ml, and samples were taken as described for the mutant. Transfer RNA was isolated as described in Materials and Methods and was used in increasing concentrations in the standard attachment assay system. These data suggest that the charging level is higher in the mutant grown in 200 μg of histidine per ml than in 100 μg/ml (Table 1). As expected, the charging level of the *hisS* mutant in 200 μg of histidine per ml is comparable to that of the wild-type strain in 100 μg of histidine per ml (Table 1).

Rate of histidyl-tRNA synthetase formation in the hisS$^+$ and hisS mutant strains during histidine-restricted and -unre-

TABLE 1. *Histidine-transfer RNA charged in vivo in SP1 and SB2449*[a]

Strain	Growth medium	Percent Charged $tRNA^{His}$
SP1	Minimal glucose plus L-histidine (100 μg/ml)	73
SB2449	Minimal glucose plus L-histidine (100 μg/ml)	24
SB2449	Minimal glucose plus L-histidine (200 μg/ml)	64

[a] In vivo charging of histidine-tRNA in a *hisS*$^+$ strain, SP1, and *hisS* mutant, SP2449. Sampling, extraction and preparation of transfer RNA were as described in Materials and Methods. The acceptance activity was determined for the control and periodate-treated samples by using the standard attachment assay system. Crude extract (15 μg) and L-[^{14}C]histidine (2×10^{-4} M) were held constant, and increasing amounts of each tRNA sample was used. The results are the average of two experiments with four determinations for each tRNA sample.

stricted growth. Figure 6 is a plot showing the differential rate of histidyl-tRNA synthetase formation in the wild-type and the *hisS* mutant strains during histidine-restricted and -unrestricted growth. After growth in unrestricted growth medium, the mutant and wild-type cells were washed twice and suspended in medium supplemented with 0.5 μg of glycyl-histidine per ml. Samples were taken during restricted growth, and at the time indicated by the arrow 100 μg of L-histidine per ml was added to the wild-type culture, and 200 μg of L-histidine per ml was added to the mutant culture. During histidine-limiting growth conditions, the differential rate of histidyl-tRNA synthetase formation in the *hisS* mutant was greater than that observed for the *hisS*$^+$ strain (Fig. 6). In contrast, during histidine-unrestricted growth, the synthesis of histidyl-tRNA synthetase in the mutant was repressed to essentially the same extent as the wild type during the first one-third to one-half mass doubling (Fig. 5). Thus, whereas growth of the *hisS* mutant in medium containing less than the K_m concentration of histidine allowed a derepressed rate of synthesis, restoration of excess histidine to this derepressed culture resulted in a rate of synthesis similar to that of the *hisS*$^+$ strain.

DISCUSSION

These data further strengthen previous findings that the synthesis of this synthetase is regulated by a repression process and that histidyl-tRNA or a product thereof is a reasonable candidate for the corepressor for histidyl-tRNA synthetase regulation (5, 10).

Growth of this *hisS* mutant in a concentration of L-histidine less than the K_m resulted in derepressed rates of formation of histidyl-tRNA synthetase and histidinol phosphate phosphatase, along with a considerable reduced level of charging (aminoacylation) of histidine tRNA. On the other hand, during growth of this *hisS* mutant in medium supplemented with concentrations of L-histidine greater than the K_m, the rate of formation of histidyl-tRNA synthetase was comparable to that of the wild-type strain in unrestricted medium, and the in vivo charging of histidine tRNA increased to a level similar to the percent charging observed for a normal strain grown in histidine-unrestricted medium.

The significant point being made in this report is that the elevated rate of histidyl-tRNA synthetase formation in the *hisS* mu-

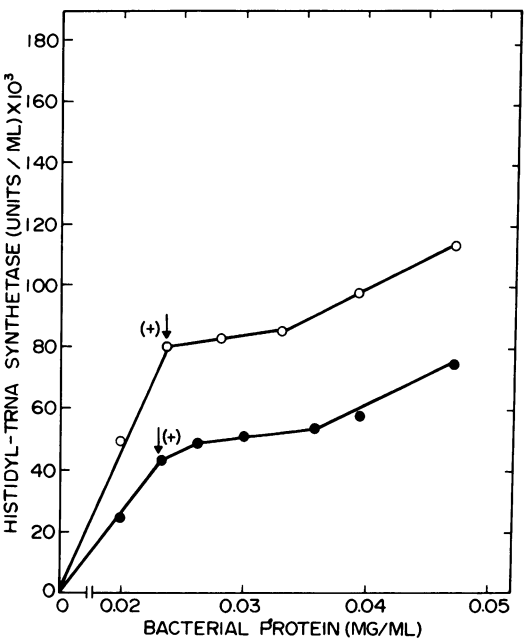

FIG. 6. *Effect of histidine-restricted and -unrestricted growth on the differential rate of formation of histidyl-tRNA synthetase in the hisS*$^+$ *and hisS mutant strains. The cells were initially grown in histidine-unrestricted medium, harvested, washed twice with minimal medium, and transferred to histidine-restricted medium (0.5 μg of glycyl-D L-histidine per ml). At the time indicated by the arrow, 100 μg of L-histidine per ml was added to the wild type, and 200 μg of L-histidine per ml was added to the hisS mutant culture. Histidyl-tRNA synthetase activity was determined for both the hisS*$^+$ (●——●) *and hisS mutant* (○——○). *Data are plotted as described in Fig. 3.*

tant, as compared to the wild-type strain, is due to the *hisS* mutation. Thus, under specific growth conditions, both derepressive and repressive responses of histidyl-tRNA synthetase formation should be evident. The results presented (Fig. 5 and 6) clearly indicate that histidine restriction caused further derepression of histidyl-tRNA synthetase formation in the *hisS* mutant and, as expected, a derepression in the *hisS*$^+$ strain. Similarly, the addition of excess histidine to both the wild-type and mutant derepressed cultures resulted in repression of synthesis of this enzyme in both cells. Therefore, the *hisS* mutant is not constitutive for histidyl-tRNA synthetase formation. Rather, these results indicate that indispensable role of histidyl-tRNA synthetase in the control of synthetase formation.

Based on the results of previous studies and those in this paper, we proposed that histidyl-tRNA or a derivative of it is the functional corepressor for regulation of histidyl-tRNA synthetase formation. This model predicts that histidine restriction of a wild-type *hisS*$^+$ and *hisR*$^+$ strain will result in derepression. Evidence to support this notion has been provided by the studies of Williams and Neidhardt (10) and McGinnis and Williams (5). The second prediction of the model is that a *hisR* (structural gene for $tRNA^{His}$) mutation would result in nonrepressible synthesis of this enzyme. McGinnis and Williams (5) have reported evidence consistent with this hypothesis. Finally, the model predicts that a *hisS* mutant possessing a reduced capacity to transfer histidine to histidine tRNA would, under specific growth conditions, exhibit a derepressed rate of histidyl-tRNA synthetase formation. The results of the present report provide evidence in support of this notion. Thus, the supply of the three units (histidine, histidine tRNA, and histidyl-tRNA synthetase) have been examined, and the results supported the idea that histidyl-tRNA is the corepressor (or repressor) for histidyl-tRNA synthetase formation.

Furthermore, other investigators have provided similar data for regulation of the histidine operon (6–8). In view of the data for histidyl-tRNA synthetase regulation, we suggest that regulation of expression of two distinct genetic units (histidine operon and histidyl-tRNA synthetase) is accomplished by means of a common effector (i.e., histidyl-tRNA) even if the regulatory response is different in sensitivity to the repression signal.

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