JOURNAL OF BACTERIOLOGY, Aug. 1978, p. 721-725
0021-9193/78/0135-0721$02.00/0

Vol. 135, No. 2
Printed in U.S.A.

Expression of the Histidine Operon in *rho* Mutants of *Escherichia coli*†

MALCOLM E. WINKLER

Department of Biology, The Johns Hopkins University, Baltimore, Maryland 21218

Received for publication 18 April 1978

Expression of the *Escherichia coli* histidine operon was measured in four independently isolated sets of strains carrying ten different *rho* mutations. Rho factor does not act as a major regulatory element of histidine operon attenuation.

Mutations in the gene for the transcription termination factor, rho, have been reported to increase tryptophan (*trp*) operon expression in *Escherichia coli* (12, 13). Specifically, rho factor has been implicated as possibly playing a role in premature messenger RNA termination at the *trp* operon attenuator in vivo (12, 13). In vitro, rho factor was not required for termination at the *trp* attenuator (15). Other studies show that another rho mutation, *suA*, increased expression of the isoleucine-valine operon (22). These combined results have led to the suggestion that rho factor-mediated terminations of transcription may be a common mechanism in the regulation of biosynthetic operons (13).

Premature mRNA termination (attenuation) has been reported to be an important factor in control of the biosynthetic histidine (*his*) operon of *Salmonella typhimurium* (11). *S. typhimurium* and *E. coli* share common mechanisms for *his* operon regulation (2, 7, 8, 14, 17; also below). Therefore, we examined the effect of a series of *rho* mutations and strong polarity suppressor mutations on *his* operon expression in *E. coli*.

Although *his* operon expression is virtually the same in the presence or the absence of exogenous histidine (1, 5), excess L-histidine (0.26 mM) was added to all cultures to maximize attenuation and possible discernment of any rho-mediated changes in attenuation. The genetic background in each set of strains was isogenic or nearly isogenic (Table 1).

his operon expression was unchanged in three of the Korn and Yanofsky *rho* mutants (strains psu 1, psu 2, and psu 3, Table 2) and was only slightly altered in two of their *rho* mutants (strains psu 4 and psu 5, Table 2). Strain psu 4 consistently showed a very small increase in HisB enzyme level, whereas strain psu 5 showed a very small decrease; however, these changes were minimal. Neither the *rho104* mutation present in strain psu 4 nor the *rho105* mutation present in strain psu 5 affected wild-type *trp* operon expression (13; C. Yanofsky, personal communication). Furthermore, strain psu 5 has not been fully characterized genetically (C. Yanofsky, personal communication). The *rho* mutations that most strongly affect *trp* operon attenuation (strains psu 1 and psu 2) (12, 13, 25) did not affect wild-type *his* operon expression. Presumably, *his* operon attenuation was unaffected by these *rho* mutations. Strain psu 2 carried both the *trpX* mutation, which mediates a $tRNA^{Trp}$ modification, and the *rho102* mutation. Because *his* operon expression was normal in strain psu 2, the *trpX* mutation apparently did not mediate modification of $tRNA^{His}$ in a way which affected regulation of *his* operon expression (see literature cited in reference 23). Under our culture conditions, this set of *rho* mutants grew faster than previously reported (12, 13). However, the strains were Trp^-, showed the correct polarity suppression of the tryptophan and lactose operons, and were temperature sensitive in minimal medium (12, 13).

Few independently isolated polarity suppressors affect *trp* operon expression (13). Therefore, we examined the effect of several other *rho* mutations on *his* operon expression (Table 3). The conditionally lethal, temperature-sensitive *rho* mutation of Das et al. suppresses many types of polar mutations, including nonsense mutations and IS1, IS2, and IS3 insertions (3). Additionally, *trp* operon expression is increased three- to fourfold by the *rho15*(Ts) mutation (S. Adhya, personal communication). This *rho* mutation did not affect *his* operon expression in bacteria grown at 32°C (Table 3A) and only slightly increased *his* operon expression in bacteria grown at 37°C (data not shown) or at 39°C (Table 3A). Both the parental strain SB4042 and the *rho* strain SB4043 possessed higher basal HisB levels than some of the other K-12 strains examined (Tables 2 and 3); similar variations have been found in different sublines of *S. typhimurium* (see literature cited in reference

† Contribution no. 956 from the Department of Biology, The Johns Hopkins University, Baltimore, MD 21218.

TABLE 1. *Bacterial strains*[a]

Strain and genotype	Source
(A) Korn-Yanofsky *rho* mutants	
Parent = W3110 *trpR trpE trpA lacZU118* Azi^r Val^r	Korn and Yanofsky (12)
psu 1 = *rho101*	Korn and Yanofsky (12)
psu 2 = *trpX rho102*	Korn and Yanofsky (12, 25)
psu 3 = *rho103*	Korn and Yanofsky (12)
psu 4 = *rho104*	Korn and Yanofsky (13)
psu 5 = *rho105*	C. Yanofsky (unpublished data)
(B) Das-Court-Adhya *rho* mutant	
SA1030 = F$^-$ *his gal*::IS3 Str^r	Das et al. (3)
AD1600 = SA1030 *rho15*(Ts)	Das et al. (3)
SB4042 = F$^-$ *gal*::IS3 Str^r	SA1030 × W3102 phage[b]
SB4043 = SB4042 *rho15*(Ts)	AD1600 × W3102 phage[b]
W3102 = F$^-$ *gal supE* Str^r λ^s	P. E. Hartman stock
(C) Malamy strong polarity suppressors	
RVNal = F$^-$ *thi ΔlacX74 nalA* Sm^s	M. H. Malamy (unpublished)
SuA115 = RVNal *rho115*	M. H. Malamy (unpublished)
SuA218 = RVNal *rho218*	M. H. Malamy (unpublished)
SuA221 = RVNal *rho221*	M. H. Malamy (unpublished)
(D) Guarente-Mitchell-Beckwith *rho* mutant	
B1 = F$^-$ *trpR ΔlacU169 rpsL thi Δ(tonB-lacP)X8605*	Guarente et al. (9, 10)
B2 = B1 *rho201*	Guarente et al. (9, 10)
B3 = F$^-$ *trpR*$^+$ *ΔlacU169 rpsL thi Δ(tonB-lacP)X8605*	Guarente et al. (9, 10)
B4 = B3 *rho201*	Guarente et al. (9, 10)

[a] All strains except those listed under (D) were placed through single-colony isolation. All strains were found to have appropriate phenotypes.

[b] W3102 phage was the P1607H phage of Wall and Harriman (21) grown on strain W3102. Transductions to *his*$^+$ were accomplished by procedures similar to those described by Miller (18).

TABLE 2. *His operon expression in the Korn-Yanofsky rho mutants*[a]

Strain	Doubling time (min)	HisB sp act (U/ml per A_{650})	HisD[b] sp act (U/ml per A_{650})	Relative sp act	
				HisB	HisD
Parent	69 ± 2	1.5 ± 0.1	0.8 ± 0.1	1.0 ± 0.1	1.0 ± 0.2
psu 1 (*rho101*)	88 ± 2	1.6 ± 0.1	0.6 ± 0.1	1.1 ± 0.1	0.8 ± 0.2
psu 2 (*trpX rho102*)	89 ± 2	1.5 ± 0.1	0.7 ± 0.1	1.0 ± 0.1	0.9 ± 0.2
psu 3 (*rho103*)	87 ± 2	1.7 ± 0.1	NT[c]	1.1 ± 0.1	
psu 4 (*rho104*)	129 ± 1	2.0 ± 0.04	1.2 ± 0.1	1.3 ± 0.1	1.5 ± 0.2
psu 5 (*rho105*)	151 ± 1	1.3 ± 0.02	NT	0.87 ± 0.06	

[a] Bacteria were grown in Vogel-Bonner minimal E medium (5) supplemented with 0.4% D-glucose, 100 μg of L-tryptophan per ml, and 0.26 mM L-histidine. Growth temperature was 39.0 ± 0.1°C. Detailed methods are presented elsewhere (23). Briefly, bacteria were grown for several mass doublings, and appropriate volumes of culture were filtered, resuspended, and frozen for assay. At least two samples were removed from each culture at different times during steady-state growth. Each sample was assayed in duplicate for HisB enzyme activity in toluenized extracts essentially as described by Ely (5). The assay for HisD enzyme activity is outlined in footnote *b*. Individual steady-state experiments were performed at least twice for each strain. The same basic results were observed for bacteria grown at 37.0°C in minimal A medium (lacking citrate) (5) supplemented with 0.4% D-glucose, 200 μg of L-tryptophan per ml, and 0.26 mM L-histidine. Standard deviation of the mean and propagated standard deviations are presented as precision indexes.

[b] HisD enzyme activity was measured in a time-course assay similar to the one used previously for phosphogluconate dehydrogenase (23). The HisD assay buffer, substrate, cofactor, and dye mix were prepared according to reference 24. Each assay tube contained 0.25 ml of assay buffer, 0.010 ml of gelatin, 0.25 ml of toluenized extract, 0.30 ml of dye mix, and 0.050 ml of substrate (water for blank tubes). The reaction was stopped by adding 0.20 ml of 0.67 M HCl. Units are in A_{520} per 60 min of assay. A variable lag sometimes preceded the linear time courses. Activities were calculated from the slope of the linear region after the lag.

[c] NT, Not tested.

23). The increased *his* operon expression with increased growth temperature in both parental and *rho* strains seemed unusual and might be due to the presence of a second temperature-sensitive mutation in this background. Strains containing other backgrounds, which were transduced to *his*$^+$ with the same phage stock, did not have similar temperature-dependent *his* enzyme

TABLE 3. *His operon expression in additional rho mutants*[a]

Strain	Temp (°C)	Doubling time (min)	HisB sp act (U/ml per A_{650})	HisD sp act (U/ml per A_{650})	Relative sp act	
					HisB	HisD
(A) Das-Court-Adhya *rho* mutant[b]						
SB4042	32.0 ± 0,1	104 ± 1	3.6 ± 0.2	NT[c]	1.0 ± 0.1	
SB4043 [*rho15*(Ts)]	32.0 ± 0.1	137 ± 2	3.8 ± 0.2	NT	1.1 ± 0.1	
SB4042	39.0 ± 0.1	70 ± 1	6.1 ± 0.4	NT	1.0 ± 0.1	
SB4043 [*rho15*(Ts)]	39.0 ± 0.1	105 ± 9	7.9 ± 0.1	NT	1.3 ± 0.1	
(B) Malamy strong polarity suppressors						
RVNal	37.0 ± 0.1	43 ± 1	1.2 ± 0.1	1.2 ± 0.1	1.0 ± 0.1	1.0 ± 0.1
SuA115	37.0 ± 0.1	56 ± 4	1.6 ± 0.1	1.8 ± 0.2	1.3 ± 0.1	1.5 ± 0.2
SuA218	37.0 ± 0.1	50 ± 2	1.3 ± 0.1	1.0 ± 0.1	1.1 ± 0.1	0.8 ± 0.1
SuA221	37.0 ± 0.1	43 ± 1	1.5 ± 0.1	1.4 ± 0.2	1.3 ± 0.1	1.2 ± 0.2
(C) Guarente-Mitchell-Beckwith *rho* mutant						
B1 (*trpR*)	37.0 ± 0.5	87 ± 3	6.0 ± 0.1	NT	1.0 ± 0.02	
B2 (*trpR rho201*)	37.0 ± 0.5	118 ± 3	9.2 ± 0.3	NT	1.5 ± 0.1	
B3 (*trpR*$^+$)	37.0 ± 0.5	93 ± 4	6.3 ± 0.1	NT	1.0 ± 0.02	
B4 (*trpR*$^+$ *rho201*)	37.0 ± 0.5	97 ± 5	12.2 ± 0.5	NT	1.9 ± 0.1	

[a] Bacteria were grown in Vogel-Bonner minimal E medium (5) supplemented with 0.4% D-glucose, 2 μg of thiamin per ml, and 0.26 mM L-histidine. Experimental details are given in footnotes *a* and *b* of Table 2.

[b] Experimental cultures were checked for their content of the *rho15*(Ts) mutation by adding 0.1 ml of each culture to 5 ml of Difco nutrient broth. Strain SB4043 failed to grow after 12 to 18 h of incubation at 43°C (3).

[c] NT, Not tested.

levels. Regardless, the effect of the *rho15*(Ts) mutation on *his* operon expression was at best slight at the three temperatures tested.

Minimal increases in *his* operon expression were found in two of three separate mutants obtained by Malamy as containing strong suppressors of insertion polarity (Table 3B). These three suppressors cause overproduction of rho factor protein (19), perhaps by a defect in rho factor-dependent attenuation of *rho* gene transcription (autoregulation) (19). Also, the molecular weight of rho protein from strain SuA221 is larger than normal (19).

Finally, we assayed two sets of strains containing the *rho201* mutation of Guarente, Mitchell, and Beckwith. One set was *trpR* (strains B1 and B2, Table 3C), whereas the other set was *trpR*$^+$ (strains B3 and B4, Table 3C). The *rho201* mutation is a powerful polarity suppressor, which allows efficient read-through of the transcription termination site at the end of the *trp* operon (10). The *rho201* mutation does not seem to affect *trp* operon attenuation (10). In the *trpR*$^+$ background, the *rho201* mutation seemed to cause a small but significant increase in *his* operon expression. However, in the *trpR* background, the effect of the *rho201* mutation on *his* operon expression was again small. *his* operon expression should be independent of the *trpR* locus (strains B1 and B3, Table 3C). Therefore, the different increases seen in the two sets of strains may represent differences in genetic backgrounds independent of the *rho* locus. Another indication of background differences was provided by the doubling times. Strain B2 grew slower than strain B1, whereas strains B3 and B4 grew at about the same rate. *rho* mutations generally decreased growth rates in minimal medium (Tables 2 and 3).

In the above experiments, HisB enzyme-specific activity (histidinol-phosphate phosphatase, EC 3.1.3.15) was used as a measure of steady-state *his* operon expression (5, 23). Sample dilutions indicated that the correction curve of *S. typhimurium* HisB activity to initial rates (5) applied equally well to *E. coli* HisB activity.

An internal (P2) promoter has not yet been clearly demonstrated operator proximal to the *hisB* gene in *E. coli*. If such a promoter exists and is substantially more active than the *S. typhimurium* P2 promoter (6), the effects of rho factor at the attenuator as judged by HisB enzyme levels might be obscured. However, in eight strains (Tables 2 and 3), changes in HisB-specific activity were paralleled by changes in HisD-specific activity (histidinol dehydrogenase, EC 1.1.1.23). The *hisD* gene is located operator proximal to both the hypothetical P2 site and the *hisB* gene (1, 8).

Further evidence against an abnormally active *his* P2 promoter in *E. coli* is provided by polarity data. Strongly polar mutations in the *hisC* or

hisD genes result in lower than wild-type levels of HisB enzyme (8). Lastly, recent data (R. Lawther and G. Hatfield, in preparation) can be interpreted as indicating the presence of an *E. coli* P2 promoter that functions at a level comparable to that of the *S. typhimurium* P2 promoter (6). Therefore, HisB enzyme activity provides a valid measure of *his* operon expression in *E. coli* strains containing at least wild-type levels of *his* operon expression. Because the HisB enzyme assay is considerably more sensitive and reliable than the colorimetric HisD enzyme assay (16), only HisB enzyme levels were measured in several of the strains.

The small increases of *his* operon enzyme levels seen in some of the *rho* mutants should be viewed cautiously. Because *rho* mutations are pleiotropic (3), such small in vivo differences could result from secondary effects. For example, the *rho15*(Ts) mutation has been reported to cause temperature-sensitive methionine auxotrophy at 42°C (S. K. Guterman and C. L. Howitt, Abstr. Annu. Meet. Am. Soc. Microbiol. 1978, H110, p. 122). At 39°C (Table 3), a partial deficiency of methionine induced by the *rho15*(Ts) mutation might result in slightly increased *his* operon expression via metabolic regulation (23).

Additionally, although absorbancy at 650 nm (A_{650}) provides a good calibration of protein concentration (23), its use in the measurement of very small differences (i.e., 1.3- to 1.4-fold) in specific activity for strains with widely varying doubling times has not been established. Finally, *rho* mutations were not detected in *S. typhimurium* during a search for mutants with increased *his* operon expression, although mutations with less than a fourfold effect might have been missed (20).

The above data and considerations suggest that if rho factor affects wild-type *his* operon expression, its effect is at best relatively small. By comparison, deletion of the *S. typhimurium* *his* operon attenuator region (mutation *his 01242*) increases *his* operon expression about 26-fold (5). Thus, rho factor apparently is not a major regulatory element of *his* operon attenuation. Consistent with this interpretation, the *Salmonella hisO* region lacks features in its DNA sequence characteristically found for absolutely rho factor-dependent terminators (W. M. Barnes, personal communication). Results similar to ours have been obtained in other laboratories (R. Lawther and G. Hatfield, in preparation; S. Adhya, personal communication; F. Blasi, personal communication; L. Korn, personal communication).

An array of phenotypes is exhibited by strains carrying different *rho* mutations. It is possible that *rho* mutations of types other than those examined in this study could affect *his* operon expression. However, the results presented here at least suggest differences in mechanism or rho specificity between *his* operon and *trp* operon attenuation. Interestingly, preliminary results imply that expression of the biosynthetic leucine operon is also unaffected in a number of *rho* mutants (4).

The *rho* gene is located very close to a histidine regulatory gene (*hisR*) on the enteric linkage map. Our results show that the two genes are distinct genes because none of the *rho* mutations elicited an HisR phenotype (1). During studies of *his* operon regulation in *rho* mutants, one must be wary of the close linkage of the *rho* and *hisR* genes. Second-site mutations in *hisR* might not be separated from *rho* mutations by transduction. Such *hisR rho* double mutants might falsely suggest an effect of *rho* on *his* operon attenuation. Should mutants with appreciable rho factor-mediated increases in *his* operon expression be found, they must be carefully examined to distinguish if the effect is due to relief of attenuation or to read-through into the operon from upstream genes (10).

I thank S. Adhya, J. Beckwith, A. Das, L. Guarente, L. Korn, M. Malamy, R. Sandri, and C. Yanofsky for supplying bacteria and phages. I also thank S. Adhya, F. Blasi, G. Hatfield, L. Korn, R. Lawther, C. Yanofsky, and W. Barnes for communicating unpublished data, Marc Rhoades for access to equipment, and Philip E. Hartman for encouragement.

This work was supported by Public Health Service grant AI01650 from the National Institute of Allergy and Infectious Diseases (to P.E.H.). M.E.W. was a trainee on Public Health Service grant HD139 from the National Institute of Child Health and Human Development.

LITERATURE CITED

1. **Brenner, M., and B. N. Ames.** 1971. The histidine operon and its regulation, p. 349–387. *In* H. J. Vogel (ed.), Metabolic pathways, vol. V. Metabolic regulation. Academic Press, Inc., New York.
2. **Bruni, C. B., V. Colantuoni, L. Svordone, R. Cortese, and F. Blasi.** 1977. Biochemical and regulatory properties of *Escherichia coli* K-12 *hisT* mutants. J. Bacteriol. **130**:4–10.
3. **Das, A., D. Court, and S. Adhya.** 1976. Isolation and characterization of conditional lethal mutants of *Escherichia coli* defective in transcription termination factor rho. Proc. Natl. Acad. Sci. U.S.A. **73**:1959–1963.
4. **Davis, M. G., and J. M. Calvo.** 1977. Relationship between messenger ribonucleic acid and enzyme levels specified by the leucine operon of *Escherichia coli* K-12. J. Bacteriol. **131**:997–1007.
5. **Ely, B.** 1974. Physiological studies of *Salmonella* histidine operator-promoter mutants. Genetics **78**:593–606.
6. **Ely, B., and Z. Ciesla.** 1974. Internal promoter P2 of the histidine operon of *Salmonella typhimurium*. J. Bacteriol. **120**:984–986.
7. **Fink, G. R., and J. R. Roth.** 1968. Histidine regulatory mutants in *Salmonella typhimurium*. VI. Dominance studies. J. Mol. Biol. **33**:547–557.
8. **Garrick-Silversmith, L., and P. E. Hartman.** 1970. Histidine-requiring mutants of *Escherichia coli* K-12.

Genetics **66**:231-244.

9. **Guarente, L. P., and J. Beckwith.** 1978. Mutant RNA polymerase of *Escherichia coli* terminates transcription in strains making defective rho factor. Proc. Natl. Acad. Sci. U.S.A. **75**:294-297.
10. **Guarente, L. P., D. H. Mitchell, and J. Beckwith.** 1977. Transcription termination at the end of the tryptophan operon of *Escherichia coli*. J. Mol. Biol. **112**:423-436.
11. **Kasai, T.** 1974. Regulation of expression of the histidine operon in *Salmonella typhimurium*. Nature (London) **249**:523-527.
12. **Korn, L. J., and C. Yanofsky.** 1976. Polarity suppressors increase expression of the wild-type tryptophan operon of *Escherichia coli*. J. Mol. Biol. **103**:395-409.
13. **Korn, L. J., and C. Yanofsky.** 1976. Polarity suppressors defective in transcription termination at the attenuator of the tryptophan operon of Escherichia coli have altered rho factor. J. Mol. Biol. **106**:231-241.
14. **Lawther, R. P., and G. W. Hatfield.** 1977. Biochemical characterization of an *Escherichia coli hisT* strain. J. Bacteriol. **130**:552-557.
15. **Lee, F., and C. Yanofsky.** 1977. Transcription termination at the *trp* operon attenuators of *Escherichia coli* and *Salmonella typhimurium*: RNA secondary structure and regulation of termination. Proc. Natl. Acad. Sci. U.S.A. **74**:4365-4369.
16. **Martin, R. G., M. A. Berberich, B. N. Ames, W. W. Davis, R. H. Goldberger, and J. D. Yourno.** 1971. Enzymes and intermediates of histidine biosynthesis in *Salmonella typhimurium*. Methods Enzymol. **178**:3-44.
17. **Martin-Del-Rio, R., V. M. Fernandez, A. R. Tebar, and A. O. Ballesteros.** 1973. Genetic characterization of histidine regulatory mutants of *E. coli* K12. Genet. Iber. **25**:29-35.
18. **Miller, J. H.** 1972. Experiments in molecular genetics. Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y.
19. **Ratner, D.** 1976. Evidence that mutations in the *suA* polarity suppressing gene directly affect termination factor rho. Nature (London) **259**:151-153.
20. **Roth, J. R., D. N. Anton, and P. E. Hartman.** 1966. Histidine regulatory mutants in *Salmonella typhimurium*. I. Isolation and general properties. J. Mol. Biol. **22**:305-323.
21. **Wall, J. D., and P. D. Harriman.** 1974. Phage P1 mutants with altered transducing abilities for *Escherichia coli*. Virology **59**:532-544.
22. **Wasmuth, J. J., and H. E. Umbarger.** 1973. Effect of isoleucine, valine, or leucine starvation on the potential for formation of the branched-chain amino acid biosynthetic enzymes. J. Bacteriol. **116**:548-561.
23. **Winkler, M. E., D. J. Roth, and P. E. Hartman.** 1978. Promoter and attenuator related metabolic regulation of the *Salmonella typhimurium* histidine operon. J. Bacteriol. **133**:830-843.
24. **Wyche, J. H., B. Ely, T. A. Cebula, M. C. Snead, and P. E. Hartman.** 1974. Histidyl-transfer ribonucleic acid synthetase in positive control of the histidine operon in *Salmonella typhimurium*. J. Bacteriol. **117**:708-716.
25. **Yanofsky, C., and L. Soll.** 1977. Mutations affecting $tRNA^{Trp}$ and its charging and their effect on regulation of transcription termination at the attenuator of the tryptophan operon. J. Mol. Biol. **113**:663-667.