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Effects of Altered *rho* Gene Product on the Expression of the *Escherichia coli* Histidine Operon

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An altered *rho* gene product affects expression of the *his* operon of *Escherichia coli*. The effect is greater for the operator proximal portion of the *his* operon than for the operator distal portion. This "*rho* effect" appears to be independent of the site of action of *hisT*-altered histidyl-tRNA.

Recent developments in the study of regulation of procaryotic gene expression have established the existence of regulation by "premature" termination of transcription at specific chromosomal sites called attenuators (4). The attenuators studied to date cause RNA polymerase to terminate before transcribing the gene(s) which follow, and their function has been found to be dependent either on the *rho* gene in vivo or on *rho* factor in vitro or both (21). Attenuation is believed to be modulated by the action of specific antiterminator molecules that relieve this transcriptional termination (4).

For example, the course of development of phage lambda is mediated not only by the lambda repressor (18) but also by *rho* factor-dependent attenuator sites which prevent RNA polymerase from transcribing the genes necessary for lytic development (21). The expression of these genes is activated by the synthesis of an antiterminator, the product of the lambda *N* gene (21). This regulation by attenuation is supported both by in vitro RNA transcription studies (20) and by the recent demonstration that, although N^- strains of lambda do not grow on wild-type strains of *Escherichia coli*, they will grow in strains altered in *rho* (7, 9, 19).

Attenuation has also been elegantly demonstrated in the tryptophan (*trp*) operon (4). Jackson and Yanofsky (10) characterized deletion mutations extending into the region between the *trp* promoter-operator region and the first structural gene of the tryptophan operon. These deletions increased the expression of the distal portion of this operon. Bertrand et al. (5) showed that the region defined by these deletions contains an attenuator site, and Korn and Yanofsky (13, 14) have shown that strains selected to relieve polarity mutations in the tryptophan structural genes also relieve attentuation and contain altered *rho* factors.

Studies by Wasmuth and Umbarger (23) and Smith et al. (22) on the regulation of the isoleucine-valine (*ilv*) genes in *E. coli* have demonstrated a derepression of the *ilv* gene activities in strains with an altered *rho* factor. By analogy to *trp* and lambda, these results have been interpreted as evidence for attenuation as a mechanism for regulation of the *ilv* genes (4, 23; R. P. Lawther and G. W. Hatfield, Mol. Gen. Genet., in press).

In the above studies, a correlation has been drawn between *rho* factor and regulation by attenuation. In the case of the histidine (*his*) operon in *Salmonella typhimurium*, it has not been possible to make this correlation because of the absence of strains with altered *rho* factor. Kasai's (11) initial proposal of attenuation of the *S. typhimurium his* operon was based upon evidence obtained with a mutant strain containing a small deletion between the promoter-operator region of the *his* operon and the first structural gene. The in vitro enzyme synthesis studies of Artz and Broach (2) have further supported the concept of an attenuator regulating the expression of the histidine operon.

We have recently constructed isogenic strains of *E. coli* containing pairwise combinations of mutations that affect the regulation of the *ilvEDA* operon (Lawther and Hatfield, in press). Two of these mutations that have been shown to alter the regulation of this operon are *rho221* (23; Lawther and Hatfield, in press) and *hisT76* (15). To ascertain that these strains contained the desired alleles, several phenotypes for each strain were monitored. For the *hisT76* allele, alteration of the leucyl-tRNA profile on reverse-phase chromatography (RPC-5) (15) and derepression of histidinol phosphatase were checked (6, 15). For *rho221*, co-transduction with *ilv*, derepression of *ilv* gene activities (23; Lawther and Hatfield, in press), suppression of a pair of *trp* polar mutations, and reduced growth rate relative to wild type (on glucose but

not on glycerol) were examined (13).

Because attenuation plays a significant role in the regulation of the *his* operon of *S. typhimurium*, it seemed possible that there would also be an increase in the expression of the *his* operon in the *rho221*-containing strains. As can be seen in Table 1, there is a small increase (1.4-fold) in the specific activity of histidinol phosphatase (product of *hisB*) in the *rho221*-containing strain (T31-4-480) relative to the wild-type strain (T31-4-4). However, the strain containing both the *hisT76* and the *rho221* mutations (T31-H-480) showed no additional increase relative to the strain containing only the *hisT76* mutation (T31-H-4).

Since the effect of the altered *rho* factor on the expression of histidinol phosphatase was small and because there may exist an internal promoter in the *his* operon of *E. coli* between *hisD* and *hisB* (comparable to that observed in *S. typhimurium*; 8) which might interfere with the observation of *rho*-mediated regulation, additional enzymes on either side of the possible internal promoter were assayed. The specific activity of histidinol dehydrogenase (product of *hisD*) was determined to measure the expression of the *his* operon before the presumed internal promoter. Again, as seen with histidinol phosphatase, there was an increase (1.7-fold) in the specific activity of histidinol dehydrogenase in the *rho221*-containing strain (T31-4-480) relative to the wild-type strain (T31-4-4). However, in contrast to the expression of histidinol phosphatase, there was also an increase (1.4-fold) in the strain containing both the *hisT76* and the *rho221* mutations (T31-H-480) relative to the strain containing only the *hisT76* mutation. Further, as shown in Table 1, the *rho221* and *hisT76* effects on the expression of histidinol dehydrogenase are additive in the double-mutant strain.

To further establish the effect of the *rho221* allele on the expression of the operator proximal portion of the *his* operon, the specific activity of ATP phosphoribosyltransferase (product of *hisG*, the first structural gene of the *his* operon) was determined. As can be seen in Table 1, there was an increase (2.3-fold) in the specific activity of the *hisG* gene product in the *rho221*-containing strain relative to the wild type, and, as was the case for histidinol dehydrogenase, an increase (1.4-fold) was observed in the strain containing both the *hisT76* and the *rho221* mutations relative to the *hisT76*-containing strain. As seen with histidinol dehydrogenase, the increases observed for the *rho221* allele and *hisT76* allele are additive in the double-mutant strain.

To further monitor the expression of the operator distal portion of the *his* operon, the specific activity of the complex of phosphoribosyl-ATP hydrolase–phosphoribosyl-AMP-1,6-cyclohydrolase (products of *hisE* and *hisI*, respectively) was determined. Clearly Table 1 shows that there was an increase (1.4-fold) in the expression of this complex in the *rho221*-containing strain, but there was no increase in the strain containing both the *hisT76* and *rho221* mutations, relative to the strain containing only the *hisT76* mutations. This pattern of gene regulation is analogous, if not identical, to that

TABLE 1. *Effect of hisT76 and rho221 mutations on the expression of the his operon*[a, b]

Strain	Relevant genotype	Enzyme activity			
		ATP-phosphoribosyltransferase[c] (*hisG*)	Histidinol dehydrogenase[d] (*hisD*)	Histidinol phosphatase[e] (*hisB*)	Phosphoribosyl-ATP-hydrolase and AMP-1-6-cyclohydrolase[f] (*hisE hisI*)
T31-4-4	Wild type	18 (1)[g]	0.337 (1)	1.24 (1)	2.64 (1)
T31-4-480	*rho221*	41 (2.3)	0.557 (1.7)	1.71 (1.4)	3.74 (1.4)
T31-H-4	*hisT76*	97 (5.4)	1.79 (5.3)	4.89 (3.9)	10.90 (4.1)
T31-H-480	*rho221 hisT76*	142 (7.9)	2.45 (7.3)	4.84 (3.9)	12.04 (4.6)

[a] Indicated strains were grown at 37°C to late log phase on M63 minimal salts (17) with 0.5% glucose, 50 μg of tryptophan, and 5 μg of thiamine hydrochloride per ml.

[b] Gene order of the *his* operon of *E. coli: OGDCBHAFIE* (3).

[c] ATP-phosphoribosyltransferase was assayed by the method of Kleeman and Parsons (12). Specific activity is in picomoles per minute per milligram of protein.

[d] Histidinol dehydrogenase was assayed by the method of Wyche et al. (25). Specific activity is in Δ A520/hr/A650.

[e] Histidinol phosphatase was assayed by the method of Ames et al. (1). Specific activity is in nanomoles per minute per absorbance at 650 nm.

[f] Phosphoribosyl-ATP-hydrolase and AMP-1,6-cyclohydrolase were assayed as a complex by combining the methods of Martin et al. (16). Specific activity is in Δ absorbance at 290 nm per minute per milligram of protein.

[g] Relative specific activity.

observed for histidinol phosphatase.

A characteristic of the *rho221* strain (T31-4-480) is a reduced rate of growth on minimal medium with glucose as sole carbon source (doubling time, 105 min) when compared to the wild-type strain (doubling time, 84 min). This difference in growth rate might reflect an imbalance in cellular metabolism, which could result in the observed increase in the specific activity of the enzymes for histidine biosynthesis. Because the wild-type strain and the *rho221* strain have identical doubling times (138 min) on minimal medium with glycerol as sole carbon source, it is possible to examine the role of a difference in growth rate on enzyme expression in these strains. If the increase in expression of the *his* operon is a function of the growth rate when these strains are grown on glucose, there should be no increase in the expression of this operon when these strains are grown on glycerol with identical doubling times. As can be seen in Table 2, there is, in fact, an increase in the expression of the *his* operon when strain T31-4-480 (*rho221*) is grown on glycerol. The histidinol dehydrogenase specific activity is increased 4.4-fold, and the histidinol phosphatase specific activity is increased 2.4-fold on glycerol, whereas on glucose, the specific activities of these enzymes are increased 1.7-fold and 1.4-fold, respectively. These data indicate that the increased expression of the *his* operon was not the result of a metabolic imbalance. If anything, these data suggest that the effect of the *rho221* allele on the *his* operon observed in Table 1 might be reduced because of the poor growth of the mutant strains in medium with glucose as a sole carbon source.

Winkler (24) has also examined the effect of altered *rho* factor on the expression of the *his* operon in *E. coli* and has concluded that altered *rho* factor does not act as a major regulatory element for the *his* operon. The question of the importance or meaning of small alterations (less than several-fold) in enzyme levels is indeed difficult to evaluate. Our approach has differed from Winkler's in several ways. First, we have chosen to introduce a *rho* allele into a genetic background in which we have established a number of defined loci, whereas Winkler has examined a broad spectrum of *rho* alleles with appropriate wild types. Because of the nature of our strains, it was also possible for us to establish the effect of the *rho221* allele relative to the *hisT76* allele. The total pattern of the data presented here leads us to the conclusion that altered *rho* factor affects in vivo expression of the *his* operon. The effects of the *hisT76* and *rho221* mutations on the expression of the operator proximal portion of the *his* operon are additive, whereas the effects of these two mutations on the operator distal portion of the *his* operon are not. This parallel behavior within the pair of proximal and pair of distal *his* gene products, rather than the changes observed for a single enzyme between strains or different enzymes within a single strain of *E. coli*, is the basis of our conclusion. Furthermore, examination of multiple enzymes within the *his* operon, in isogenic strains, ensures that the observed differences are not due to an artifact of a single gene product or caused by interference by a non-*his* gene product. Also, because of the detectable difference between the effect of the *rho221* allele and the *hisT76* allele, it is reasonable to assume that the alterations of *rho* factor and histidyl-tRNA affect different sites in the *his* operon.

TABLE 2. *Effect of growth on glycerol on expression of the his operon in strains T31-4-4 and T31-4-480*[a]

Strain	Relevant genotype	Enzyme activity	
		Histidinol dehydrogenase (*hisD*)	Histidinol phosphatase (*hisB*)
T31-4-4	Wild type	0.882 (1)[b]	2.24 (1)
T31-4-480	*rho221*	3.86 (4.4)	5.35 (2.4)

[a] Indicated strains were grown at 37°C to late log phase on M63 minimal salts (17) with 2.0% glycerol plus 50 μg of thiamine-hydrochloride per ml.

[b] Relative specific activity.

As indicated elsewhere for the *ilvEDA* operon (Lawther and Hatfield, in press), perturbations of gene regulation caused by altered *rho* factor may not indicate a real role for *rho* factor in the expression of a particular gene. It is not inconceivable for an altered *rho* factor to interfere with the response of DNA-dependent RNA polymerase to non-*rho* punctuation in the genome.

Whether, in fact, an internal promoter in the *his* operon of *E. coli* is responsible for the observed differences in the expression of the proximal and distal portions of the *his* operon is not readily established. Such an internal promoter is the only known element within the *his* operon that might be responsible for this effect. It seems premature to propose any additional regulator elements or sites for this operon at this time.

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