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Internal Promoter P2 of the Histidine Operon of *Salmonella typhimurium*[1]

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The histidine operon internal promoter P2 allows initiation of transcription at a rate up to one-third that of the wild-type *his* operon.

The histidine operon of *Salmonella typhimurium* consists of nine contiguous genes coding for the histidine biosynthetic enzymes (8). Normal transcription of the *his* operon is initiated within a control region (*hisO*) located at one end of the operon (6) and results in the production of a single, multicistronic messenger ribonucleic acid for the entire operon (2, 7). Atkins and Loper (1) have demonstrated that transcription also can be initiated within the structural genes of the *his* operon. Two internal promoters, designated P2 and P3, have been located by complementation analysis using strains containing deletions of the *hisO* region (1). P2 was found to be operator proximal to the *hisB* gene, and P3 was found to be operator proximal to the *hisI* gene (Fig. 1).

Since the *hisB* enzyme is often measured as an indicator of the level of *his* operon expression (5, 9, 13), we attempted to determine what contribution P2 makes to the expression of the *hisB* gene. The contribution of P2 can be determined by comparing the level of expression of a gene preceding P2, the *hisD* gene, to that of the distal *hisB* gene. Data presented below indicate that the internal promoter P2 allows initiation at transcription at a rate up to one-third of the rate of the wild-type *his* operon, and that P2 transcription can account for almost the entire level of *hisB* gene expression in some strains where transcription of the *hisD* gene is nearly eliminated.

All strains used in this study have been described elsewhere (5, 6). Growth of cells for assays has been described by Wyche et al. (13). Histidinol phosphate phosphatase (*hisB* enzyme) activity was assayed as described by Ely (5). Histidinol dehydrogenase (*hisD* enzyme) was assayed by the method of Cieslà et al. (4).

hisD and *hisB* enzyme levels are presented in Table 1 for a number of strains isogenic except for the *hisO* region. A control strain containing *his-515* was deleted for the entire *his* operon and showed no detectable *hisD* or *hisB* enzyme activities. Strains containing the promoter-like mutations *hisO2321* or *hisO2355* had specific activities of 0.03 and 0.02 U, respectively, for the *hisD* enzyme, less than 3% of the wild-type level. In contrast, *hisB* enzyme levels for these strains were 0.4 and 0.5 U, respectively, or approximately 30% of the wild-type level. Since the *hisD* enzyme results only from those initiations occurring in this *hisO* region, whereas *hisB* enzyme results from the combined expression of *hisO* and P2, we conclude that virtually all of the *hisB* enzyme activity found in these strains is due to expression of P2.

Strains containing one of three additional promoter-like mutations, *hisO2965*, *hisO2966*, or *hisO3148*, had specific activities of approximately 0.1 U for the *hisD* enzyme and 0.6 U for the *hisB* enzyme in the presence of excess histidine. In this case, transcription originating in this *hisO* region would be expected to make a contribution of approximately 0.1 U of activity to the *hisB* enzyme levels. Since the differences between the level of *hisB* and *hisD* gene expression is 0.4 to 0.5 U, we conclude that the level of expression of the internal promoter P2 in these strains is the same as that found for the two strains discussed above.

In the absence of histidine, strains containing *hisO2355*, *hisO2965*, *hisO2966*, or *hisO3148* exhibit derepression (5). Table 1 shows a parallel increase in both *hisD* and *hisB* enzyme levels, indicating coordinate expression of the *his* operon under these conditions. However, the ratio of *hisD* to *hisB* enzyme levels in these strains approaches that found for strains with a higher level of *his* operon expression (*his*$^+$ and

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hisO3150). If the rate of P2 expression were constant regardless of the rate of transcription originating at *hisO*, we would expect the ratio of *hisD* to *hisB* activities to increase with increasing specific activities until the effect of P2 became insignificant at levels of specific activities greater than 5 U. For instance, transcription initiated at P2 would contribute 30% of the enzyme activity when the *hisB* enzyme level was 1.5 U but only 10% when the *hisB* enzyme level was 5.0 U. Instead, the *hisD*-to-*hisB* enzyme ratio seemed to reach a maximum when *hisD* enzyme levels reached a specific activity of approximately 1.0 U. Thus, these results suggest that transcription initiated at *hisO* interferes with transcription initiations beginning at P2 and that the contribution of P2 expression to *hisB* enzyme levels is negligible when strains have a wild-type, or higher, level of *his* operon expression. Similar observations of a primary promoter interfering with expression of an internal promoter have been made in the tryptophan operon by Morse and Yanofsky (10, 11) and by Callahan and Balbinder (3).

Since P2 expression is negligible in strains containing at least a wild-type level of *his* enzymes, the assay of *hisB* enzyme activity can provide a true measure of *his* operon expression in these strains. On the other hand, when *his* operon expression is reduced to a level significantly below that of wild type, expression beginning at P2 increases and makes a major contribution to the level of the *hisB* enzyme. Therefore, the *hisD* enzyme assay should be used to measure low levels of *his* operon expression. P2 does not affect the expression of the *hisD* gene, and the *hisD* enzyme assay is about 250 times more sensitive (4). If the *hisB* enzyme assay is used for strains with a low level of *his* operon expression, a correction for the contribution of P2 must be made.

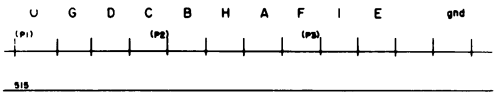

FIG. 1. *His operon of S. typhimurium (adapted reference 8).*

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LITERATURE CITED

1. Atkins, J. F., and J. C. Loper. 1970. Transcription initiation in the histidine operon of *Salmonella typhimurium*. Proc. Nat. Acad. Sci. U.S.A. **65**:925-932.
2. Brenner, M., and B. N. Ames. 1971. The histidine operon and its regulation, p. 349-387. *In* H. J. Vogel (ed.), Metabolic pathways, vol. 5. Academic Press Inc., New York.
3. Callahan, R., III, and E. Balbinder. 1970. Tryptophan operon: structural gene mutation creating a "promoter" and leading to 5-methyltroptophan dependence. Science **168**:1586-1589.
4. Cieslà, Z., F. Salvatore, J. R. Broach, S. W. Artz, and B.

TABLE 1. *Comparison of hisD and hisB enzyme levels in strains containing mutations at the primary his operon promoter*

his mutation	0.1 mM L-histidine added to growth medium			No histidine addition		
	hisD[a] (U/OD$_{650}$)	*hisB*[b] (U/OD$_{650}$)	*D/B* ratio	*hisD*[a] (U/OD$_{650}$)	*his*[b] (U/OD$_{650}$)	*D/B* ratio
his-515	<0.0004	<0.1		NG[c]	NG[c]	
his02321[d]	0.03	0.4	0.08	NG	NG	
his02355[d]	0.02	0.5	0.04	0.56	1.3	0.4
his02965[d]	0.11	0.6	0.2	0.82	1.4	0.6
his02966[d]	0.12	0.6	0.2	0.71	1.5	0.5
his03148[d]	0.10	0.6	0.2	0.99	1.5	0.7
his03149[d]	0.82	1.6	0.5	1.3	2.2	0.6
his03150[d]	4.1	5.2	0.8	4.4	6.2	0.7
his$^+$	1.4	1.4	1.0	1.6	2.2	0.7

[a] *hisD* enzyme activities are the average of two or more determinations, with each determination performed in duplicate.

[b] *hisB* enzyme activities are the average of two or more determinations, with each determination performed in duplicate.

[c] NG, No growth.

[d] *hisO* mutations used in this study are "promoter-like" mutations that lie in between constitutive mutations in the *hisO* region (6). A description of their effects on *his* operon expression can be found in reference 5.

N. Ames. 1974. Histidine regulation in *Salmonella typhimurium*. XVI. A sensitive radiochemical assay for histidinol dehydrogenase. Anal. Biochem., vol. 62.
5. Ely, B. 1974. Physiological studies of *Salmonella* histidine operator-promoter mutants. Genetics, vol. 78.
6. Ely, B., D. B. Fankhauser, and P. E. Hartman. 1974. A fine structure map of the *Salmonella* operator-promoter. Genetics, vol. 78.
7. Goldberger, R. F., and J. S. Kovach. 1972. Regulation of histidine biosynthesis in *Salmonella typhimurium*. Curr. Top. Cell. Reg. **5**:285–308.
8. Hartman, P. E., Z. Hartman, R. C. Stahl, and B. N. Ames. 1971. Classification and mapping of spontaneous and induced mutations in the histidine operon of *Salmonella*. Advan. Genet. **16**:1–34.
9. Lewis, J. A., and B. N. Ames. 1972. Histidine regulation in *Salmonella typhimurium*. XI. The percentage of transfer RNA^{His} charges *in vivo* and its relation to the repression of the histidine operon. J. Mol. Biol. **66**:131–142.
10. Morse, D. E., and C. Yanofsky. 1968. The internal low-efficiency promoter of the trytophan operon of *Escherichia coli*. J. Mol. Biol. **38**:447–451.
11. Morse, D. E., and C. Yanofsky. 1969. A transcription-initiating mutation within a structural gene of the trytophan operon. J. Mol. Biol. **41**:317–328.
12. Roth, J. R., D. N. Antón, and P. E. Hartman. 1966. Histidine regulatory mutants in *Salmonella typhimurium*. I. Isolation and general properties. J. Mol. Biol. **22**:305–323.
13. 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.