JOURNAL OF BACTERIOLOGY, Feb. 1983, p. 1114–1119
0021-9193/83/021114-06$02.00/0

Vol. 153, No. 2

Internal Promoters of the *his* Operon in *Salmonella typhimurium*

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Received 13 September 1982/Accepted 29 November 1982

Two internal promoters in the *his* operon of *Salmonella typhimurium* have been precisely mapped genetically. The internal promoters are found in, or very close to, gene border regions in the *his* operon. The *his* operon was examined for the presence of additional internal promoters whose transcripts were sensitive to *rho*-mediated transcription termination and therefore had escaped detection. No new internal promoters were found. It is argued that the internal promoters described here are not likely to be fortuitous message start sites, but may play a physiologically important role in operon expression.

Internal promoters appear to be quite common in large bacterial operons (2, 3, 6, 9, 14), but their role is not understood. They could allow regulation of the operon in a noncoordinate manner, allowing different levels of gene products in the operon or temporally different expression of certain genes. Alternatively, their major role could be to reinforce expression of downstream genes, which would otherwise be transcribed in lower amounts owing to the effects of natural polarity. It is also possible that these promoters are physiologically unimportant and are merely fortuitously occurring sequences able to act as low-level promoters. To approach the study of such promoters, we have accurately mapped their location in the histidine operon.

The histidine biosynthetic operon of *Salmonella typhimurium* consists of nine genes that are regulated coordinately. All of these genes are transcribed from a primary promoter (P1), which is subject to attenuation control (1, 15, 17). Two other functional promoters exist within the *his* operon (2, 7) that are capable of transcribing portions of the operon. These internal promoters have been roughly mapped previously but have never been precisely located (2, 7, 19, 22).

The general location of the *his* internal promoters was first described by Atkins and Loper (2) and has been confirmed by others (7, 19, 22). Expression originating from the internal promoters was observed by eliminating the primary promoter (P1) by deletion (2, 7, 22) or by blocking P1 transcripts with a Tn*10* insertion (19). The P2 promoter then allows expression of the *hisB*, *H*, *A*, *F*, *I*, and *E* genes. The P2 promoter was previously mapped to a region including the last half of the *hisC* gene and the first half of the *hisB* gene (2, 20). Another internal promoter (P3) allows expression of the *hisI* and *hisE* genes and can be seen when the P1 and P2 promoters are removed. A genetic map showing the *his* operon and location of the internal promoters is presented in Fig. 1. The precision of the mapping by Atkins and Loper (2) was limited by the number of available deletions that removed the P1 or both the P1 and P2 promoters; the precision of mapping by Kleckner and co-workers (19, 20) was limited by the number and location of available Tn*10* insertion mutations.

The experiments described here rely on a Tn*10* insertion to block transcription from the major *his* promoter P1, thus allowing the presence or absence of downstream gene expression to be absolutely correlated with the presence or absence of the internal promoter. The Tn*10* element itself has a promoter that is able to transcribe sequences outside of the inserted element (4), but these Tn*10*-initiated transcripts are efficiently terminated by the *rho*-dependent termination mechanism and do not express downstream genes under the conditions used (4). Experimentally, expression arising from a *his* internal promoter is easily differentiated from expression arising from a Tn*10* promoter.

Genetic mapping of the first internal promoter, P2. Previous work on the P2 promoter has placed it within a region including the last half of the *hisC* gene and the first half of the *hisB* gene, as shown in Fig. 1. To refine the location of P2, strains were constructed that have *hisG8570*::Tn*10* (which blocks transcripts from P1) and an internal *his* deletion. Strains used are listed in Table 1. In an otherwise wild-type strain, this *hisG*::Tn*10* insertion prevents expression of the *hisG*, *D*, and *C* genes; *hisB* and

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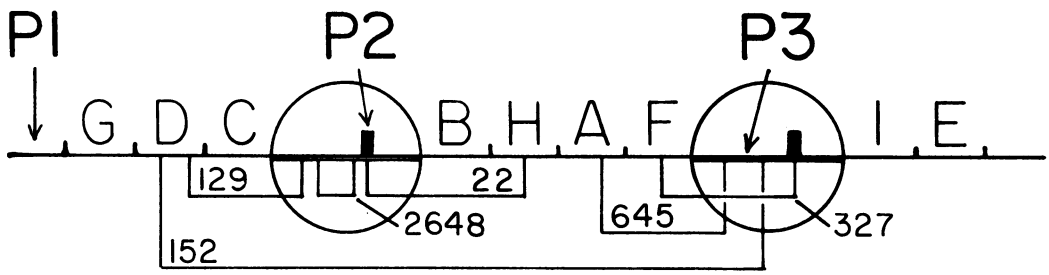

FIG. 1. A genetic map of the *his* operon of *S. typhimurium*. P1, P2, and P3 indicate the map locations of the three known promoters in the *his* operon.

TABLE 1. Multiply marked bacterial strains

Strain	Genotype[a]	Source
TR35	*his-712 ser-821 arg-501*/F' T80 *his*$^+$	Roth and Fink, unpublished data
TR51	*trpA8 purE801 his-612*/F' T80 *hisD2381*	Roth and Fink, unpublished data
TR53	*his-712 ser-821 arg-501*/F' T80 *hisC2383*	Roth and Fink, unpublished data
TR75	*his-712 ser-821 arg-501*/F' T80 *hisB2405*	Roth and Fink, unpublished data
TR76	*his-712 ser-821 arg-501*/F' T80 *hisA2406*	Roth and Fink, unpublished data
TR78	*his-712 ser-821 arg-501*/F' T80 *hisF2408*	Roth and Fink, unpublished data
TR82	*his-712 ser-821 arg-501*/F' T80 *hisI2412*	Roth and Fink, unpublished data
TR83	*his-712 ser-821 arg-501*/F' T80 *hisIE2413*	Roth and Fink, unpublished data
TA34	*his-712 ser-821*/F' T80 Δ(*hisHAFIE*)	Roth and Fink, unpublished data
TG5701	*his-712 ser-821 arg-501*/F' T80 *hisG2416*	R. F. Goldberger
TT428	*hisG8570*::Tn*10*	Roth collection
TT1157	*hisA8697*::Tn*10*	Roth collection
TT2789	*his-22 phoN2 zee-1*::Tn*10*	T. Kohno
TT2790	*his-22 phoP12 zee-1*::Tn*10*	T. Kohno
TT4156	*hisG8570*::Tn*10 phoN2*	This study
TT4157	*hisG8570*::Tn*10 phoP12*	This study
TT4647	*hisG8575*::Tn*10 his-152*	This study
TT4648	*hisG8575*::Tn*10*	This study
TT4776	*hisG8570*::Tn*10 his-22*	This study
TT4777	*hisG8570*::Tn*10*	This study
TT4778	*hisG8570*::Tn*10 his-129*	This study
TT4779	*hisG8570*::Tn*10*	This study
TT4784	*hisH8677*::Tn*10 his-327*	This study
TT4785	*hisH8677*::Tn*10*	This study
TT4786	*hisH8677*::Tn*10 his-645*	This study
TT4787	*hisH8677*::Tn*10*	This study
TT6702	*hisG8570*::Tn*10 his-2648 hisT1504*	This study
TT6703	*hisG8570*::Tn*10 hisT1504*	This study
TT7295	*rho*$^+$ *his-57 ilvA595*::Tn*10*	This study
TT7296	*rho-111 his-57 ilvA595*::Tn*10*	This study
TT7297	*rho*$^+$ *hisO9615 hisG1102 hisT1504*	This study
TT7298	*rho-111 hisO9615 hisG1102 hisT1504*	This study
TT7416	*his-646 zee-1*::Tn*10 phoP12*	This study
TT7417	*his-646 zee-1*::Tn*10 phoN2*	This study
TR5691	*hisT1504 his-2648*	B. N. Ames
TR6214	*ilvE401 metE338 his01242 hisC2124 trpE49 amtA1 ara-9 rho*$^+$	Housley et al. (12)
TA2361	*phoN29*	B. N. Ames
TA2362	*phoP12*	B. N. Ames

[a] All F' T80 episomes are *his*$^+$ for all *his* genes except the specific genes mentioned in the genotype.

subsequent genes are transcribed from P2. If the tested *his* deletion removes the P2 promoter, then *hisB*, *H*, *A*, and *F* will not be expressed. If the *his* deletion does not remove the P2 promoter, then *hisB*, *H*, *A*, and *F* will be expressed. In either case, the genes *hisI* and *E* will still be expressed, owing to the P3 internal promoter. Gene expression was measured by complementation, using a series of *Escherichia coli* F′ *his* episomes carrying various well-characterized *his* mutations on the episome. Data critical to the final placement of P2 are shown in Table 2. It is concluded that deletion *his-129* does not affect the P2 promoter, deletion *his-22* removes or severely damages P2, and deletion *his-2648* damages but does not remove the promoter (see Fig. 1).

P2 promoter directs expression of *hisB* phosphatase. The endpoint of *his-22* is very close to the *hisC-hisB* gene border. There are no *hisC* point mutations known to be deleted by *his-22*, and there are no *hisB* point mutations known to lie outside deletion *his-22* (11). The *hisB* enzyme is a single polypeptide encoding two distinct enzymatic functions: a phosphatase and a dehydratase. The regions encoding these two activities are clearly separated on the *hisB* genetic map; the phosphatase activity is encoded in the promoter proximal region of *hisB*, whereas the dehydratase activity is located in the promoter distal region, near *hisH* (13; T. Kohno and B. Cooper-Kohno, personal communication).

It has been found that a nonspecific acid phosphatase, encoded by the *phoN* gene, unlinked to the *his* operon and normally uninvolved in *his* biosynthesis, can substitute for the *hisB* phosphatase activity (T. Kohno, manuscript in preparation). Thus, mutations eliminating only the phosphatase activity of the *hisB* gene product are suppressed by the *phoN*$^+$ gene, and strains containing such *hisB* mutations are not detected as *his* auxotrophs. Mutations in either the *phoP* or *phoN* gene abolish the suppressing nonspecific phosphatase activity (18; Kohno and Cooper-Kohno, personal communication). Strains carrying either of these *pho* mutations cannot grow on minimal medium without a functional *hisB* phosphatase (Kohno, personal communication). Both *hisB* complementation and *hisB* phosphatase enzyme levels produced by the P1 or P2 promoters were measured in strains which carry either the *phoP* or *phoN* mutation. These data are shown in Table 2, lines 10 and 11, and in Table 3. From these data, it can be concluded that P2 must direct expression of the *hisB* phosphatase, since strains lacking both the major *his* promoter (P1) and the *pho* phosphatase retain *hisB* phosphatase complementation and enzyme activity. The location of the P2 promoter must be within, or at the end of, deletion *his-22* but must precede the operator-proximal phosphatase segment of the *hisB* gene. This limits P2 to a tiny region including the intercistronic punctuation sites at the *hisC-hisB* border (see Fig. 1).

Genetic mapping of the second internal promoter, P3. The general location of the P3 promoter was determined by Atkins and Loper (2) and by

TABLE 2. Location of the P2 promoter

Genotype	Complementation of F′ T80 *his* episomes[a]								
	his$^+$	*G*	*D*	*C*	*B*	*A*	*F*	*IE*	*ΔHAFIE*
his-129	+	+	−	−	+	+	+	+	+
hisG::Tn*10 his-129*	+	−	−	−	+	+	+	+	+
hisG::Tn*10*	+	−	−	−	+	+	+	+	+
his-22	+	+	+	+	−	+	+	+	−
hisG::Tn*10 his-22*	+	−	−	−	−	∓	∓	+	−
hisG::Tn*10*	+	−	−	−	+	+	+	+	+
his-2648	+	+	+	−	+	+	+	+	+
hisG::Tn*10 his-2648*	+	−	−	−	±	+	+	+	−
hisG::Tn*10*	+	−	−	−	+	+	+	+	+
hisG::Tn*10 phoN*	+	−	−	−	+	+	+	+	+
hisG::Tn*10 phoP*	+	−	−	−	+	+	+	+	+

[a] Complementation data with F′ T80 *his* episomes with either a *his*$^+$ operon (TR35) or a *his* mutation, as listed across the top of the table. The recipient strains are grouped into isogenic sets. The data were gathered initially by spot tests and, for any ambiguous or critical results, repeated with a full plate test. A + indicates that good complementation was observed within 24 to 36 h at 37°C. A ± indicates that complementation was observed within 48 to 72 h, but that it was weaker than that scored with a +. A ∓ indicates that barely detectable complementation was observed after 48 to 72 h; the growth response was never strong, even after extended incubation. Pairs scored as − showed no detectable complementation.

TABLE 3. Expression of *hisB* phosphatase by P2[a]

Strain	Phenotype	P1	P2	*rho*	Activity
LT2	His^+	+	+	+	2.36
TA2361	His^+, $PhoN^-$	+	+	−	2.06
TA2362	His^+, $PhoP^-$	+	+	−	1.92
his-646	$HisOGD^-$	−	+	+	0.32
TT428	HisG::Tn*10*	−	+	+	0.58
TT4156	HisG::Tn*10*, $PhoN^-$	−	+	−	0.42
TT4157	HisG::Tn*10*, $PhoP^-$	−	+	−	0.48
TT7416	$HisOGD^-$, $PhoN^-$	−	+	−	0.22
TT7417	HisOGD, $PhoP^-$	−	+	−	0.33
his-22	$HisBH^-$	−	−	+	0.05
TT2789	$HisBH^-$, $PhoN^-$	−	−	−	0.0
TT2790	$HisBH^-$, $PhoP^-$	−	−	−	0.0

[a] Assay levels are the average of two experiments, except in the case of TT2789 and TT2790, which were performed only once. Columns 3 and 4 indicate which *his* promoters are transcribing the *hisB* gene, whereas column 5 indicates the presence (+) or absence (−) of the *phoN* phosphatase activity. The assays were performed on toluenized cells as described by Martin et al. (20a).

Kleckner et al. (19, 20), who placed P3 within the *hisF* gene. To precisely map this promoter, we applied the same methods that were used to map the P2 promoter. A Tn*10* insertion in either *hisB*, *hisH*, or *hisA* was used to block transcription from both the P1 and P2 promoters. Normally, a strain carrying one of these Tn*10* insertions will complement *hisI* and *hisE* mutations, because of the P3 promoter. These Tn*10* insertions were combined with deletions having endpoints within *hisF*. If the deletion in question removes P3, then the *hisI* and *hisE* genes will not be expressed; if the deletion mutant retains P3, then the *hisI* and *hisE* genes will be expressed and mutations in these genes will be complemented. Table 4 shows the complementation data that test for expression of the *hisI* and *hisE* genes for the deletions critical for placing P3. It is concluded that deletions *his-327* and *his-152* remove P3, whereas deletion *his-645* leaves P3 intact (see Fig. 1). The last two deletion intervals in *hisF* are genetically quite small; if the number of point mutations is taken as an indicator of physical distance, the P3 promoter must be within the last 4% of the *hisF* gene (11).

Absence of additional internal promoters in the *his* operon. One would expect that most fortuitous message starts within operons would result in transcripts which are promptly terminated by *rho* factor, which is known to cause termination of untranslated messages (8). This has recently been shown to be true in the *his* operon in which the outward-directed promoters of Tn*10* are usually not detected, owing to early *rho*-dependent termination (4). The only internal message starts that might be detectable are those that happened to be located so near a ribosome binding site that no *rho*-dependent termination site intervened; such promoters would generate transcripts which would be protected from termination and could express distal genes. The two promoters described here are near the ends

TABLE 4. Location of the P3 promoter

Genotype	Complementation of F′ T80 *his* episomes[a]								
	his$^+$	*D*	*C*	*B*	*A*	*F*	*I*	*IE*	Δ*HAFIE*
hisF327	+	+	+	+	+	−	+	+	−
hisH::Tn*10 his-327*	+	+	+	+	−	−	−	−	−
hisH::Tn*10*	+	+	+	+	−	−	+	+	−
his-645	+	+	+	+	+	−	+	+	−
hisH::Tn*10 his-645*	+	+	+	+	−	−	+	+	−
hisH::Tn*10*	+	+	+	+	−	−	+	+	−
his-152	+	−	−	−	−	−	+	+	−
hisG::Tn*10 his-152*	+	−	−	−	−	−	−	−	−
hisG::Tn*10*	+	−	−	+	+	+	+	+	+

[a] Data are presented as in Table 2. Some of the pairs scored as − here actually have extremely weak complementation activity that is due to transcription arising from the Tn*10* element (4). In all cases, however, this level of complementation was clearly distinguishable from complementation due to the presence of P3. All F′ T80 episomes carry a mutation in the indicated *his* gene; all other *his* genes are *his*$^+$.

TABLE 5. Effect of *rho* on *his* complementation[a]

Genotype	Complementation of F' T80 *his* episomes								
	his$^+$	*G*	*D*	*C*	*B*	*A*	*F*	*IE*	*ΔHAFIE*
his-9615 rho$^+$	+	−	−	−	+	+	+	+	+
his-9615 rho	+	−	−	−	+	+	+	+	+
his-57 rho$^+$	+	−	−	−	−	−	−	+	−
his-57 rho	+	−	−	−	−	−	−	+	−

[a] Data are presented as in Table 2. Mutation *his-9615* is a phenotypically His$^-$ mutation at the left end of the *hisO* region; it is thought be a deletion of the promoter since it fails to recombine with a large series of promoter mutants. The deletion leaves all structural genes of the operon intact (16). All F' T80 episomes carry a mutation in the indicated *his* gene; all other *his* genes are *his*$^+$.

of genes, so the possibility was entertained that they might be fortuitously occurring message start sites that were detectable owing to their location near ribosome initiation sites. If this were the case, one would expect that many other internal message starts might exist which are only detectable in the absence of *rho* factor. We sought these cryptic message starts by looking for internal *his* promoters in strains carrying a *rho* mutation. By making use of transductional linkage between *rho* and *ilv*, strains were constructed that contained a *rho* mutation (*rho-111* [12]) and a *his* deletion that removed P1 or both P1 and P2. The ability of these strains to express *his* genes was tested by complementation to determine whether Rho$^-$ strains initiated transcripts at sites other than the previously described internal promoters. As seen in Table 5, the complementation pattern of these deletions in Rho$^+$ and Rho$^-$ strains is identical. Thus, within the regions checked by this test, no additional promoters are in evidence. The failure to find cryptic internal promoters encourages us to believe that fortuitous message starts within operons are rare and that the existence of P2 and P3 may reflect a selectively significant role for these promoters.

Several other lines of evidence support the selective importance of internal promoters. Winkler et al. (22) have reported that the *his* P2 promoter is subject to ppGpp control and is activated by shift-down growth conditions. Thus, this promoter seems to be under metabolic control. Evolutionary evidence supports the significance of internal promoters in both the *his* and *trp* operons. In both *S. typhimurium* and *E. coli*, internal promoters are found within the *trpD* gene (3, 14), as well as near the *hisB* gene (P2) (10). Like the *his* promoters described here, the *trp* promoter is located at the distal end of the *trpD* gene, near an intercistronic region. The conservation of these internal promoters by both *S. typhimurium* and *E. coli* is striking, since DNA sequencing shows that coding sequences differ by 15 to 25% in the *trp* operons of these two bacteria (5, 21). It seems likely that these internal promoters have been maintained by selective pressure.

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