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Cloning and Expression of the Distal Portion of the Histidine Operon of *Escherichia coli* K-12

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The operator-distal genes *hisBHAFI(E)* of the *Escherichia coli* K-12 histidine operon were mapped on a DNA fragment 4,500 base pairs long. This fragment, originally present in a λ transducing phage, was cloned in the vector plasmid pBR313. A restriction map was determined, allowing identification of the orientation of the genes in the fragment. The cloned genes were expressed in appropriate hosts, independent of the orientation of the DNA fragment, as shown by transformation tests and by enzyme assays of one of the gene products, *hisB*, histidinol phosphatase. An internal transcription initiation site was identified by isolation of the cellular RNA, hybridization to specific DNA probes, and mapping by S1 nuclease.

With the development of recombinant DNA technology, the histidine operon of the enterobacteria *Escherichia coli* and *Salmonella typhimurium* has been the subject of extensive studies (9). Most of the work has been concerned with the elucidation of the mechanisms of operon regulation. Expression of this biosynthetic operon in both species is regulated at the transcriptional level by an overall mechanism termed attenuation (9, 48). Recent studies from our and other laboratories have been concerned with the genetic analysis of the regulatory region (28), DNA sequencing of the regulatory region (4, 20, 44) and of regulatory mutants (29), and cloning and expression (transcription and translation) of the proximal part of the operon (5, 14, 23). There are several other aspects of the *his* operon organization which are of potential interest: internal promoters (1, 21), intercistronic regions (40), and multifunctional gene products (42). As a prerequisite to studying some of these features, we report here the cloning of the distal portion of the *E. coli* K-12 *his* operon, a restriction map of this region, the orientation and expression of its genes, and the mapping of an internal transcription initiation site.

MATERIALS AND METHODS

Bacterial strains, phage, and plasmids. Bacterial strains used are listed in Table 1. Strain FB251 was constructed by selecting a spontaneous *thy* mutant with thymidine-trimethoprim selection (36) in strain FB186 and mating this derivative with an Hfr strain *thy*$^+$ *recA56* (N1200), selecting for *thy*$^+$ recombinants, and scoring for UV sensitivity (14). Phage lysates and transduction tests were performed as previously described (2). Details of the construction of histidine recombinant plasmids are given below.

Media, growth conditions, and enzyme assays. Liquid media were LB broth (36) and minimal medium (45) supplemented with 0.5% glucose. Solid media contained 1.2% agar (Difco) and were nutrient broth (36) and minimal medium (45) supplemented with 0.5% glucose. Amino acids were added at 0.5 mM; L-histidine was added at 0.1 mM, and histidinol was added at 1.0 mM. Tetracycline and ampicillin were added to both liquid and solid media at 25 and 50 μg/ml, respectively. Strains for enzyme assays or for RNA preparation were grown in minimal medium to an absorbancy at 650 nm of 0.8. Assay procedures for the enzymes of the histidine pathway (histidinol phosphatase [E.C. 3.1.3.15] [B enzyme] and imidazolylacetol-phosphate:L-glutamate aminotransferase [E.C. 2.6.1.9] [C enzyme]) have been described elsewhere (34). Proteins were measured by the method of Lowry et al. (32).

Chemicals and enzymes. All amino acids, histidinol, histidinol phosphate, and glyoxal were obtained from Sigma Chemical Co. Acrylamide, methylenebisacrylamide, ammonium persulfate, *N,N,N',N'*-tetramethylethylenediamine, and agarose were from Bio-Rad Laboratories. Guanidine hydrochloride was from Bethesda Research Laboratories. [γ-^{32}P]ATP (3,000 Ci/mmol) was from Amersham Corp. or was prepared from $^{32}P_i$ by the method of Maxam and Gilbert (35); the latter was kindly donated by R. Di Lauro. Restriction endonucleases *Hpa*I, *Pst*I, *Sal*I, *Sma*I, and *Hae*II were purchased from Bethesda Research Laboratories. *Hin*dIII, *Bam*HI, and *Hin*fI were from New England Biolabs. *Bgl*II was purified as described (8). Calf intestine alkaline phosphatase was from Boehringer Mannheim Corp.; T4 polynucleotide kinase and ligase were from Miles Laboratories, Inc.; S1 nuclease was from Sigma. Either unlabeled or ^{32}P-labeled pBR322 DNA, digested with *Hin*fI, was used as a standard of molecular weight on acrylamide gels.

*Hin*dIII digests of λ DNA were used as molecular-weight standards on agarose gels.

DNA fractionation and labeling. Phage DNA (16) and plasmid DNA (17) were prepared as described elsewhere. For screening large numbers of transformants, plasmid DNA was purified by the rapid method described elsewhere (18). The cloned *Hin*dIII 4,500-base-pair (bp) fragment was isolated on and purified from preparative 1% agarose slab gels by the method of Vogelstein and Gillespie (46). DNA fragments from acrylamide gels were recovered by the method of Maxam and Gilbert (35). 5'-Terminal labeling of DNA fragments was performed with T4 polynucleotide kinase and [γ-^{32}P]ATP, as described elsewhere (35), except that calf intestine alkaline phosphatase was used instead of bacterial alkaline phosphatase and the enzyme was inactivated by heating for 10 min at 75°C.

Restriction maps. The restriction map of the plasmid DNA was determined by using restriction sites uniquely present in the vector or cloned DNA, as described below. Fine mapping of the *Hin*dIII 4,500-bp fragment was performed by the technique of Smith and Birnstiel (41), as previously described (14).

Cloning and transformation. Plasmids pVG2 and pVG3 were obtained by mixing the λ *his* DNA and the pBR313 DNA, both restricted with *Hin*dIII. The DNA mixtures were ligated and used to transform strain FB251, as previously described (14). Plasmids pVG4 and pVG5 were constructed as described below.

RNA preparation and S1 mapping. Total RNA was extracted from *E. coli* cells by the guanidine hydrochloride procedure described elsewhere (26) and modified as follows. A 200-ml amount of cultures grown in minimal medium to an absorbancy at 650 nm of 0.8 was immediately poured over five crushed ice cubes (10 ml/cube) of a killer solution (minimal medium containing 10 mM NaN_3 and 200 μg of chloramphenicol per ml) and centrifuged at 5,000 rpm for 15 min. The pelleted cells were suspended in 3 ml of 25% sucrose in 50 mM Tris-hydrochloride (pH 8.0) and left 10 min on ice. A 0.6-ml amount of lysozyme, 10 mg/ml in water, was added, followed after 10 min by 1.2 ml of 0.25 M EDTA, pH 7.0. Spheroplasts were collected by centrifugation for 15 min at 5,000 rpm and either immediately processed or frozen in dry ice-ethanol and stored at −80°C. The pellet was suspended in 5 ml of 8 M guanidine hydrochloride–50 mM sodium acetate (pH 5.0)–1 mM dithiothreitol and homogenized in a Polytron homogenizer for 2 min at maximum speed, keeping the vessel in a dry ice-ethanol bath at −50°C. The homogenate was extracted with 10 ml of chloroform-isoamyl alcohol (24:1) by homogenizing as above for 1 min and was then centrifuged. The aqueous phase was precipitated with 2.5 ml of absolute ethanol and kept at −20°C for 30 min; the precipitate was then collected by centrifugation. The precipitate was redissolved in 5 ml of 8 M guanidine hydrochloride–20 mM EDTA (pH 7.0)–1 mM dithiothreitol; sodium acetate (pH 5.0) was then added at 50 mM (final concentration) followed by 2.5 ml of absolute ethanol, and the solution was kept at −20°C for 30 min and then centrifuged. This last step was repeated three times. The pellet was suspended in 2.5 ml of 20 mM EDTA (pH 7.0), by periodic blending in a Vortex mixer and heating at 37°C for 5 to 10 min. The suspended pellet was then extracted by blending in a Vortex mixer for 2 min with 1 volume of chloroform-butanol (4:1). The suspension was centrifuged, and the organic phase was extracted once with a ½ volume of 20 mM EDTA, pH 7.0. The two aqueous phases were combined; 3 volumes of 4 M sodium acetate (pH 6.0) were added and left at −10°C overnight. The RNA was collected by centrifugation and suspended in water, and the concentration and purity were checked by the absorbancy ratio at 260 and 280 nm. The nucleic acid was precipitated again with 2.5 volumes of absolute ethanol at −70°C for 30 min, collected by centrifugation, redissolved in water at a concentration of 2 mg/ml, and stored at −80°C. The integrity of the RNA preparations was routinely checked by agarose slab gel electrophoresis of samples denatured with glyoxal by the method of Carmichael and McMaster for evaluating the relative amounts of 23 and 16S RNA (15).

RNA-DNA hybridizations, S1 nuclease digestion, and analysis of hybrids on denaturing gels were performed as described elsewhere (22). The reaction mixtures contained 200 ng of 5'-labeled DNA probe (specific activity, 500,000 cpm/μg) and different amounts of specific RNA, whereas the total concentration in each hybridization mixture was kept at 40 μg of RNA by adding nonspecific rat liver RNA. S1 nuclease digestion was for 1 h at 37°C with 100 U of enzyme per assay. Products were analyzed on 5% acrylamide slab gels in 7 M urea and visualized by autoradiography using Kodak X-Omat R films exposed at −80°C with intensifying screens.

RESULTS

λ *hisBHAFI(E)* transducing phage. This phage was originally constructed by Borck et al. (11) by cloning total *E. coli* DNA restricted with

TABLE 1. *E. coli* K-12 strains[a]

Strain	Genotype	Source or construction
FB1	Δ(*hisGDCBHAFIE*) *750 gnd rhaA*	P. E. Hartman (24)
FB8	Wild-type *E. coli* K-12, UTH1038	T. Kasai (30)
FB169	*hisB463* (λ *cI857 S7* [*hisBpBHAFIE*])	This paper and Bruni et al. (14)
FB181	*hisI903*	Goldschmidt et al. (25)
FB182	*hisF892*	Goldschmidt et al. (25)
FB184	*hisA915*	Goldschmidt et al. (25)
FB186	*hisB855*	Goldschmidt et al. (25)
FB190	*hisG2743 recA56*	Bruni et al. (14)
FB251	*hisB855 recA56*	This paper
FB252	*hisB855 recA56* (pVG2)	Transformation of FB251
FB253	*hisB855 recA56* (pVG3)	Transformation of FB251
FB254	*hisB855 recA56* (pVG4)	Transformation of FB251
FB255	*hisB855 recA56* (pVG5)	Transformation of FB251
FB256	Δ(*hisGDCBHAFIE*) *750 gnd rhaA* (pVG2)	Transformation of FB1
N1200	HfrKL16 *thr ilv recA56* Spc^r	J. L. Rosner

[a] Genetic symbols are as given by Bachmann and Low (3).

*Hin*dIII into a λ vector and isolating His$^+$ transductants in an auxotrophic *hisB* strain. Figure 1A shows the restriction pattern of the phage DNA with *Hin*dIII. The cloned fragment migrated as a 4,500-bp band slightly above the band corresponding to the right arm of the phage DNA which is normally associated with the 23,000-bp fragment comprising the left arm (Fig. 1A, lane 3) and dissociated upon heating (Fig. 1A, lane 4). Since the cloned fragment contained an asymmetric *Bgl*II restriction site (see below), we determined the orientation of the cloned fragment by digesting the phage DNA with *Bgl*II. Figure 1B shows the structure of the recombinant phage with the pertinent restriction sites. The phage was originally found by transduction tests to complement mutations in the *hisB*, *hisH*, and *hisA* genes (11). We extended such analyses and found that the phage can complement not only *hisB* and *hisA*, but also *hisF* and *hisI* mutants. It appears, therefore, that the fragment comprises most or all of the distal part of the operon. The presence of the most distal gene, *hisE*, could not be determined for lack of appropriate mutants in *E. coli*, although an equivalent fragment of *S. typhimurium* DNA does contain this gene (unpublished data).

Construction and restriction mapping of recombinant plasmids. The phage DNA and vector plasmid pBR313 (10) DNA were restricted with *Hin*dIII and ligated, and the mixture was used to transform a *hisB recA* host in the presence of ampicillin. pBR313, rather than pBR322, was chosen as the vector to facilitate subsequent purification of the inserted DNA, thanks to the different size of the molecules (Fig. 2, lanes 1

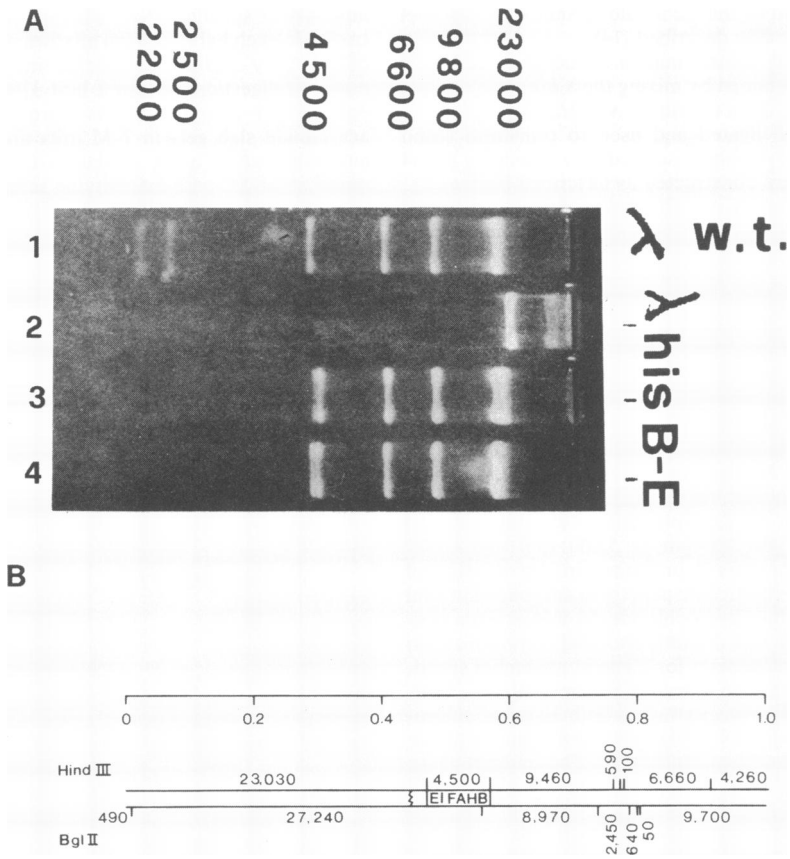

FIG. 1. Restriction pattern and map of the λ histidine transducing phage. (A) One percent agarose slab gel electrophoresis of λ *his* phage DNA digested with *Hin*dIII. Lane 1, λ Wild-type DNA used as marker. The size in bp of the bands is indicated. Lane 2, λ *his* DNA undigested. Lane 3, λ *his* DNA digested with *Hin*dIII and not heated. Lane 4, λ *his* DNA digested with *Hin*dIII and heated for 5 min at 70°C before loading the sample on the gel. It is now more evident that the 4,500-bp band comprises two fragments. The upper one is the cloned insert, and the lower one corresponds to the right arm of the phage DNA. (B) Physical map of the λ *hisBpBHAFIE* phage. The λ vector has *Eco*RI fragment B deleted, between 0.444 and 0.543 map units (top line) of the genome (⧘). The position of the restriction sites and the size of the fragments in bp are indicated for *Hin*dIII (top) and *Bgl*II (bottom). The position and gene order of the inserted *Hin*dIII *his* fragment are shown. Coordinates of sites and the size of λ wild-type fragments are as given by Davis et al. (19).

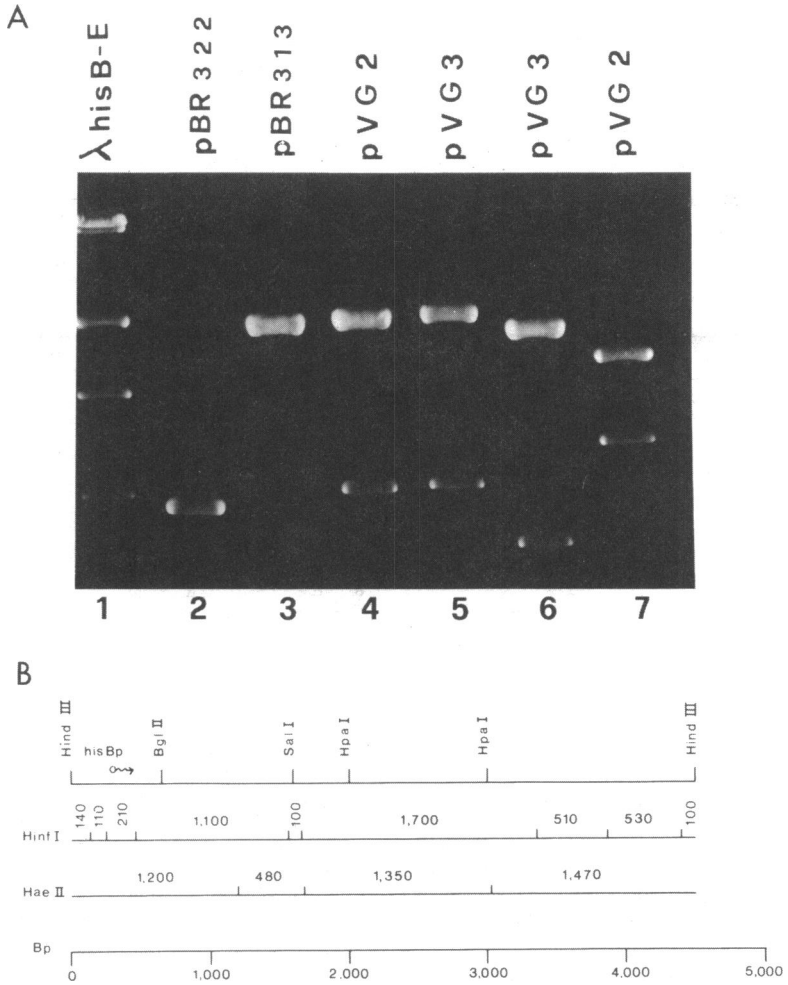

FIG. 2. Restriction pattern of *his* recombinant plasmid DNA and restriction map of the cloned *Hin*dIII 4,500-bp fragment. (A) One percent agarose slab gel of pVG2 and pVG3 DNA digested with *Hin*dIII or *Hpa*I. Lanes 1, 2, and 3, λ *his*, pBR322, and pBR313 DNAs, respectively, digested with *Hin*dIII and used as markers. Lanes 4 and 5, pVG2 and pVG3 DNAs digested with *Hin*dIII exhibit the same digestion pattern. Lanes 6 and 7, pVG3 and pVG2 DNA digested with *Hpa*I have a different restriction pattern due to the opposite orientation of the cloned fragment within the vector. (B) Restriction map of the *his* operon distal part. The top line shows the sites of restriction endonucleases which recognize 6-bp sequences. The position of the internal transcription site mapped by S1 nuclease is indicated (○⇝). The other enzymes used are indicated on the left. The different fragments are separated by vertical bars, and their size in bp is indicated on top.

through 3). The double selection (His^+ Amp^r) allowed easy isolation of the desired recombinant plasmids. Two classes of recombinant molecules, whose prototypes were named pVG2 and pVG3, were found. Upon isolation of the DNA and analysis of the restriction products on 1% agarose slab gels, we found that they show the same pattern when digested with *Hin*dIII (Fig. 2, lanes 4 and 5). Digestion of the two DNAs with *Hpa*I, however, generated different-sized fragments (Fig. 2, lanes 6 and 7), indicating that the cloned piece was inserted and expressed in the two possible orientations. An internal 1,000-bp *Hpa*I fragment is not visible in this picture. The DNA of the two plasmids was digested with several other restriction endonucleases, and *Sal*I and *Bgl*II sites were mapped in the inserted fragment. No sites for *Pst*I, *Bam*HI, or *Sma*I were present. Figure 3 shows a restriction map of the two plasmids carrying the inserted DNA in opposite orientations. A more detailed restriction map of the cloned fragment was determined. Plasmid pVG2 DNA was digested with *Hin*dIII, and the fragment was purified from preparative horizontal agarose slab gels (46). The fragment was terminally labeled with T4 polynucleotide kinase and [γ-^{32}P]ATP (35) and digested with *Sal*I, and the two 5′-labeled

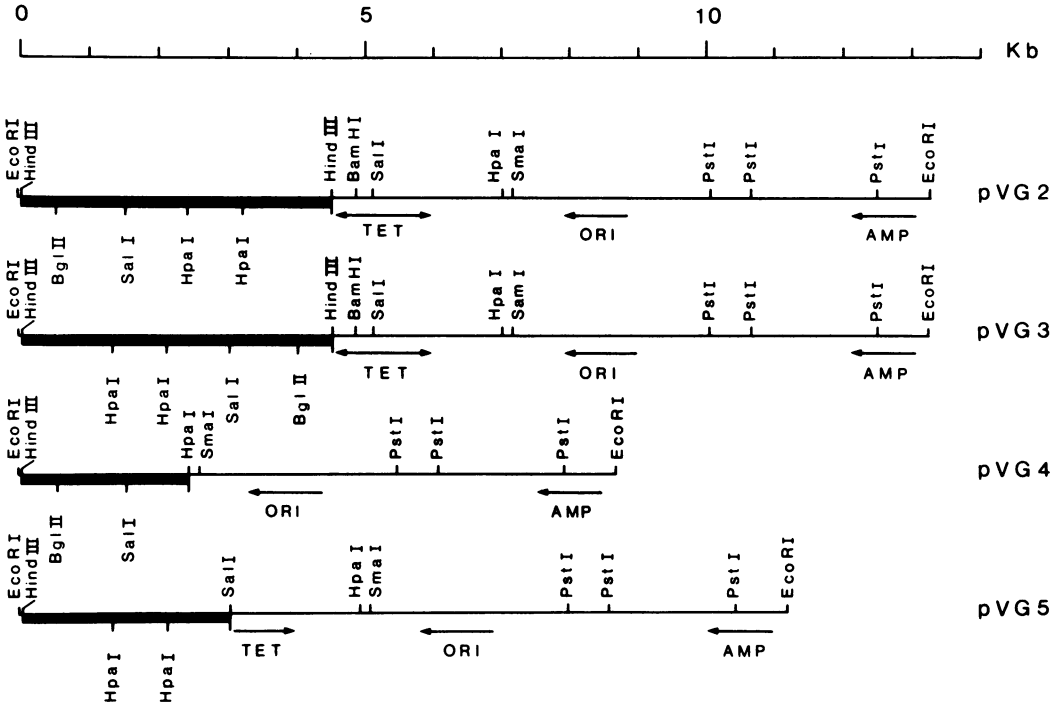

FIG. 3. Physical map of the recombinant histidine plasmids. The circular plasmid DNAs are schematically linearized at the *Eco*RI site of vector pBR313 at map position 0 megadaltons (10). The filled bars represent fragments cloned. The arrows represent the orientation of the tetracycline-resistant genes (TET), the origin of replication (ORI), and the ampicillin-resistant gene (AMP). Plasmids pVG2 and pVG3 contain the 4,500-bp DNA fragment carrying the *hisBHAFIE* distal genes inserted in the unique *Hin*dIII site of vector pBR313. They differ in the orientation of the fragment (see the text). Cells containing these plasmids were tetracycline sensitive and ampicillin resistant and expressed the *his* genes. Plasmid pVG4, also expressing the *hisB* function, contained the 2,000-bp *Hin*dIII-*Hpa*I proximal fragment with respect to the pVG2 map. Plasmid pVG5 contained the *Sal*I-*Hin*dIII distal fragment with respect to the pVG2 map. Details of the construction are given in the text.

fragments, 1,600 and 2,900 bp long, were separated and purified on acrylamide slab gels (35). The restriction map was determined as described above (Fig. 2B). The two plasmids, pVG2 and pVG3, were used to transform several *his* auxotrophic strains on minimal ampicillin medium. Again, as for the original transducing phage, prototrophic transformants were obtained in an *hisB*, *hisA*, *hisF*, and *hisI* background, but not in an *hisG* background.

Orientation of genes. To establish the orientation of the *his* genes with respect to the restriction map, we used recombinant plasmids pVG2 and pVG3, which carry the cloned fragment inserted in opposite orientations, (Fig. 3). Plasmid pVG2 DNA was digested with *Hpa*I and religated, and the mixture was restricted with *Bam*HI to eliminate pVG2-type molecules. Similarly, pVG3 DNA was digested with *Sal*I and religated, and the mixture was restricted with *Bam*HI to eliminate pVG3-type molecules. The two DNAs were used to transform *E. coli* FB251 (*hisB855 recA56*) on LB ampicillin plates. Transformants were screened for growth on minimal ampicillin plates. pVG2, but not pVG3, derivatives were found to grow on minimal plates. The DNA from representative clones of both types of transformants was isolated and characterized by restriction mapping. The restriction maps of these derivative plasmids (pVG4 and pVG5) are shown in Fig. 3. Plasmid pVG4, which was able to transform to prototrophy the *hisB* strain, contained the part of the cloned fragment from the *Hin*dIII to the first *Hpa*I site, 2,000 bp long. We therefore conclude that the *hisB* gene is contained on this piece of DNA and that the *his* genes are ordered on the fragment in a clockwise orientation, *hisB* through *hisE*, with respect to the pVG2 restriction map, *Bgl*II being the more proximal site.

Expression of the cloned genes. The fact that the *E. coli* late *his* genes are expressed in both the λ *his* transducing phage and the plasmids bearing the fragment in opposite orientation raises the possibility that the genes are transcribed from an internal promoter. A low-level constitutive promoter, proximal to the *hisB* gene, has been, in fact, shown to exist in *S.*

typhimurium (1, 21, 47). Alternatively, one has to assume that the genes are expressed under a phage promoter and plasmid promoters active in the two orientations. The tetracycline promoter lying just distal to the *Hin*dIII site (43) was inactivated in our recombinant plasmids pVG2 and pVG3, since cells transformed by these plasmids were tetracycline sensitive. To investigate this point, we performed enzyme assays on several strains harboring the phage and the plasmids. The results are reported in Table 2. The product of the *hisC* gene, not present in the cloned fragment (14), was used to establish the activity of the chromosomal genes expressed under the primary *hisGp* promoter. One of the activities of the *hisB* gene, histidinol phosphatase, was used to estimate the levels of expression of the cloned genes. The *hisB* strains used as recipients still exhibited some phosphatase activity, since the mutation affected the other enzyme function, imidazole-glycerol phosphate dehydratase, of this multifunctional gene product. The cloned *his* distal genes were expressed at a low level in the lysogenic strain harboring the λ *his* phage (strain FB169 in Table 2). The levels of histidinol phosphatase rose severalfold in the strains harboring a plasmid carrying the fragment in either orientation (strains FB252 and FB253 in Table 2). Moreover, the level of *hisB* gene expression attained in these two strains over the chromosomal *hisC* gene was very similar. Finally, the same high level of expression was found in a strain with a complete deletion of the *his* operon, harboring plasmid pVG2 (strain FB256 in Table 2). These results again suggest that the distal *his* genes are expressed from an internal promoter.

Mapping of the transcription initiation site. To obtain direct evidence for such a promoter, we used the technique of mapping RNA-DNA hybrids with the single-strand-specific S1 nuclease (7). The restriction map and gene order (see above) allowed us to predict which DNA strand is used as template and is complementary to the *his* mRNA. The pVG2 DNA was digested with *Bgl*II, labeled with T4 polynucleotide kinase and [γ-^{32}P]ATP at the 5′ ends, and redigested with *Hin*dIII. The 650-bp *Hin*dIII-*Bgl*II fragment (Fig. 2B) should have the complementary strand labeled at the 5′ position and can be used to map any internal transcription site. Since we were interested in studying the in vivo expression of *his* mRNA, we developed a method of RNA purification derived from a widely used technique to isolate large mRNA from eucaryotic cells (26), which is described above. Total RNA isolated from several strains was hybridized to the labeled probe, digested with S1 nuclease, and analyzed on denaturing acrylamide slab gels (Fig. 4). In wild-type strains, most of the mRNA was transcribed from the primary *hisGp* promoter, as shown by the complete protection of the 650-bp DNA probe (Fig. 4, lanes 5 through 7), although some RNA molecules were initiated at a secondary transcription site located 300 bp from the *Hin*dIII site. This internal site was the major one used in *hisB* strains harboring the plasmid (Fig. 4, lanes 2 through 4) and the only one used in a complete *his* deletion strain carrying the same plasmid (Fig. 4, lanes 8 through 10). These results establish the existence and location of an internal promoter, *hisBp*, in the *E. coli* *his* operon.

DISCUSSION

In previous work, we have reported the cloning and expression of the proximal part of the histidine operon of *E. coli* K-12 (13, 14) and the sequence determination and in vivo and in vitro transcription of the regulatory region (20, 23, 44). Since our aim is to analyze in more detail the overall structure of this biosynthetic operon, we report in this paper the cloning of the distal region of the operon. The length of the proximal part of the operon, comprising the entire regulatory region, the first two structural genes (*hisGD*), and the first 600 bp of the third structural gene (*hisC*), was found to be approximately 3,300 bp and to be contained in an *Hin*dIII fragment of 5,300 bp (14). If the two *Hin*dIII fragments, the 5,300-bp fragment and the one cloned here 4,500 bp long, represent contiguous

TABLE 2. Levels of histidine biosynthetic enzymes in *E. coli* K-12 strains

Strain	Genotype	Enzyme level[a]		
		C enzyme	B enzyme	C/B ratio[b]
FB8	Wild-type *E. coli* K-12, UTH1038	1.0	3.5	3.5
FB169	*hisB463* (λ cI857 *S7* [*hisBpBHAFIE*])	3.0	6.0	2.0
FB251	*hisB855 recA56*	1.2	2.9	2.4
FB252	*hisB855 recA56* (pVG2)	1.3	13.2	10.1
FB253	*hisB855 recA56* (pVG3)	1.7	15.3	9.0
FB256	Δ(*hisGDCBHAFIE*) *gnd rhaA* (pVG2)	<0.1	14.7	—[c]

[a] Enzyme-specific activities are expressed as described by Martin et al. (34).
[b] B enzyme levels are normalized to the C enzyme levels, arbitrarily set equal to 1.0.
[c] —, None.

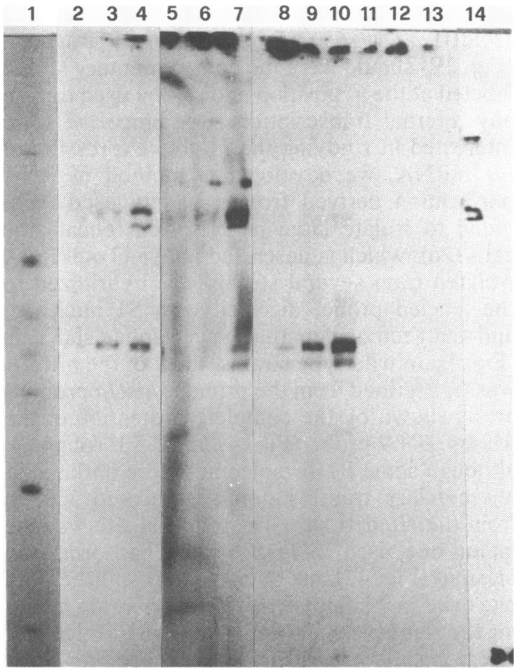

FIG. 4. S1 mapping of the internal transcription initiation site. Autoradiogram of a 5% denaturing acrylamide slab gel. Lane 1, ^{32}P-labeled pBR322 DNA digested with *Hin*fI and used as a marker of molecular weight. The size in bp of the fragments from the top is 1,633, (517 and 506), 396, 344, 298, (221 and 220), and 154. Lanes 2, 3, and 4: 5, 15, and 40 μg of RNA extracted from strain FB252 (*hisB855 recA56* [pVG2]) were hybridized to the labeled DNA probe, as described in the text. Two DNA-RNA-protected hybrids were present; the slower one comigrated with the labeled 650-bp probe (lane 14), the faster migrated as a 350-bp band. Lanes 5, 6, and 7, the same amounts of RNA extracted from strain FB251 (*hisB855 recA56*) were processed as described above. Also, in this case, the slower- and faster-migrating hybrids were present, although the faster one was present in much lesser amounts. Lanes 8, 9, and 10, RNA extracted from strain FB256 (Δ*his-750* [pVG2]) and processed as above shows only the faster-migrating hybrid. Lanes 11, 12, and 13, RNA extracted from the complete *his* deletion strain FB1 did not hybridize to the DNA probe. Lane 14, DNA probe 5'-labeled at the *Bgl*II site and cleaved with *Hin*dIII. Two bands, corresponding to the 3,850- and 650-bp fragments, were present. For reasons that are unclear, the 650-bp fragment migrated as a double band. The same gel was autoradiographed for different times: lanes 1 and 5 through 7 were autoradiographed for 90 h; lanes 2 through 4 and 8 through 14 were autoradiographed for 30 h.

segments of the bacterial chromosome, the overall length of the *his* operon should not exceed 8,000 bp. Previous estimates of the operon length, based on measurements of the molecular weights of the gene products and on transduction frequencies (12), were larger (10,000 bp) and need to be reevaluated. Particularly puzzling is the estimated length of the *hisB* primary gene product (molecular weight, 97,000, or about 900 amino acids [42], that is, 2,700 bp). If this were the case, the remaining five genes, *hisHAFIE*, should be contained in approximately 2,000 bp of genetic material. Availability of the clones will help resolve these questions.

Another aspect of the *his* operon organization which has been analyzed in this paper is whether the distal genes are also expressed from an internal transcription initiation site. We have clearly shown that this site exists and maps approximately 300 bp from the proximal end of the fragment. A similar site had previously been identified in *S. typhimurium* by complementation analysis in *E. coli-S. typhimurium* diploid hybrid strains (1) and by enzyme assays of strains carrying deletions of the primary *hisGp* promoter (21). This internal promoter was found to be subjected to metabolic regulation but not to histidine-specific regulation (47). In *E. coli* its presence was inferred from the discoordinate activity of the proximal and distal gene products in *rho*-deficient strains (31). At variance with previous reports (21), this promoter seems to function even in the presence of an efficient *hisGp* promoter. In fact, an active *hisBp* promoter could be demonstrated even when the RNA was extracted from cells grown in minimal medium, a condition in which the primary *hisGp* promoter is functioning. An internal low-efficiency constitutive promoter has also been found in the *trp* operon of *S. typhimurium* (6) and *E. coli* (37) by deletion analysis and enzyme assays and mapped at the end of the structural gene *trpD* (27, 39). The fact that internal promoters are found in several operons and in different species (39) raises the possibility that they might serve a physiological, although as-yet-undefined, role and not just be promoter-like structures (27). One possibility is that distal genes in the operons have a dual function in the cell and that the internal promoters would allow the synthesis of these gene products even in the absence of a functional primary promoter. For instance, the *hisH* and *hisF* gene products have been implicated in the mechanism of cell division (38). In this report, we provide direct evidence that an internal promoter exists and that mRNA molecules are initiated at this site. We would then expect to find in the cell not just one single *his*-specific polycistronic mRNA molecule, but at least two and possibly three (1). Interestingly, in the initial report of a polycistronic mRNA molecule corresponding to the *his* operon of *S. typhimurium*, Martin (33) found two peaks, one at 16 and one at 34S. We are currently investigating this and other aspects of *his* operon expression and organization.

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