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Nucleotide Sequence of the Gene Encoding the Repressor for the Histidine Utilization Genes of *Pseudomonas putida*

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The *hutC* gene of *Pseudomonas putida* encodes a repressor which, in combination with the inducer urocanate, regulates expression of the five structural genes necessary for conversion of histidine to glutamate, ammonia, and formate. The nucleotide sequence of the *hutC* region was determined and found to contain two open reading frames which overlapped by one nucleotide. The first open reading frame (ORF1) appeared to encode a 27,648-dalton protein of 248 amino acids whose sequence strongly resembled that of the *hut* repressor of *Klebsiella aerogenes* (A. Schwacha and R. A. Bender, J. Bacteriol. 172:5477–5481, 1990) and contained a helix-turn-helix motif that could be involved in operator binding. The gene was preceded by a sequence which was nearly identical to that of the operator site located upstream of *hutU* which controls transcription of the *hutUHIG* genes. The operator near *hutC* would presumably allow the *hut* repressor to regulate its own synthesis as well as the expression of the divergent *hutF* gene. A second open reading frame (ORF2) would encode a 21,155-dalton protein, but because this region could be deleted with only a slight effect on repressor activity, it is not likely to be involved in repressor function or structure.

Gene expression in microbial histidine utilization (Hut) systems is controlled by a number of mechanisms that respond to changes in growth conditions. Positive regulation by the cyclic AMP (cAMP)–catabolite gene activator protein (CAP) complex has been observed in some species (19, 24), but succinate-provoked carbon catabolite repression of *hut* genes in *Pseudomonas putida* and *Pseudomonas aeruginosa* occurs by a poorly understood mechanism that is not dependent on intracellular cAMP levels (22). Positive regulation in response to the nitrogen source also occurs (23).

In addition to these global regulatory mechanisms, *hut*-specific regulation is accomplished by either positive or negative regulatory proteins. In *Bacillus subtilis*, all of the *hut* genes belong to a single operon which is controlled by a positive regulator (15, 20). In *Klebsiella*, *Salmonella*, and *Pseudomonas* spp., the *hut* genes are organized into multiple transcriptional units and are under negative control by a single repressor protein, the *hutC* gene product (4, 10, 16, 28). In these organisms, urocanate, the product of the first step of the pathway, serves as the inducer for all of the *hut* genes. Furthermore, in *Pseudomonas putida*, the *hutG* gene, which encodes the last enzyme of the pathway, also has the ability to be induced by its substrate, *N*-formylglutamate (12). This has led to the proposal by Hu et al. that the *hut* repressor is a bifunctional molecule capable of binding two different operator sequences and two inducer molecules (12, 13). Evidence that the *P. putida hut* genes are regulated by a single repressor has been provided by the generation of mutants that produce all of the *hut* enzymes constitutively (12, 16) as well as mapping by subcloning into *Escherichia coli* (8, 13).

It has been shown recently (11) that the *P. putida hut* repressor binds to three different regions of DNA corresponding to potential regulatory sites for the *hutUHIG*, *hutF*, and *hutG* transcripts. The site preceding *hutG* showed much weaker binding than the other two sites and required a higher concentration of inducer (urocanate or *N*-formylglutamate) to relieve binding. The apparent *hutF* regulatory site is in the region between the divergent *hutF* and *hutC* (repressor) transcripts (13), but it has not been demonstrated directly that the *hut* repressor uses this site to regulate its own synthesis as well as that of *hutF*.

Physical mapping of the *hutC* region has been accomplished by transposon Tn*1000* insertion mutagenesis and subcloning studies (13). These studies indicated that *hutC* lies within a 1.7-kilobase (kb) region flanked on the upstream end by an *Eco*RI site and on the downstream end by a *Sal*I site.

In this report we show that the nucleotide sequence of the region of DNA previously reported to contain the gene (*hutC*) for the *hut* repressor (13) contains two open reading frames that are potentially involved in regulation of *hut* genes, although only the first of these is required for in vitro binding at the *hutUHIG* operator site. Also, a probable repressor-binding site exists in the region immediately upstream from the start of the first open reading frame, suggesting the ability of the *hut* repressor to regulate its own synthesis.

MATERIALS AND METHODS

Strains and plasmids. The cloned DNA used in this study was originally from *P. putida* PRS1 (ATCC 12633). Molecular cloning of the *hut* genes was described earlier (8). *E. coli* strains used as hosts for recombinant plasmids are shown in Table 1. The strains RDP210 and RDP145 were used to propagate pBR322 derivatives. TB1 was used as the host for the pUC8-derived constructions. The host for all pPL-lambda derivatives was N4830.

The construction of plasmid pMC4, which contains the genes for urocanase, histidase, and the *hut* repressor, was described previously by Consevage et al. (8).

Enzymes and chemicals. Restriction enzymes were purchased from New England BioLabs or International Biotechnologies Inc. T4 ligase, DNA polymerase I (Klenow fragment), and ultrapure agarose were purchased from In-

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TABLE 1. Bacterial strains and plasmids

Strain or plasmid	Description	Source or reference
E. coli		
RDP210	F⁻ *lacY1 leuB6 thi-1 hsdR hsdM rpsL supE44*	R. D. Porter
RDP145	F⁻ *galK2 hsdR4 endA1 sbcB15 tonA rpsL gyrA recAB*	R. D. Porter
TB1	Δ(*lac-pro*) *strA ara thi-1* φ80d *lacZ*ΔM15 *hsdR4*	Boehringer Mannheim
N4830	F⁻ *sup his strA recA1 galOP3 ilvA*Δ8 (λ*c*I857 ΔBAM ΔH1)	Pharmacia
Plasmids		
pBR322	Cloning vector (Ap^r Tc^r)	3
pUC8	Cloning vector (Ap^r)	29
pMC4	*hutC*$^+$ *hutU*$^+$ *hutH*$^+$	8
Subclones derived from pMC4		
pSA1	2.7-kbp *Cla*I-*Eco*RV fragment inserted into pBR322 cut with *Cla*I and *Eco*RV	This study
pSA5	1.0-kb *Sal*I fragment inserted into pBR322 cut with *Sal*I	This study
pSA7	1.4-kb *Bam*HI-*Sal*I fragment inserted into pBR322 cut with *Bam*HI and *Sal*I	This study
pSA12	0.5-kb *Bam*HI fragment inserted into pUC8 cut with *Bam*HI	This study
pSA13	1.5-kb *Hpa*I-*Ssp*I fragment inserted into pPL-lambda cut with *Hpa*I (*hutC* under control of p_L promoter)	This study
pSA14	Same as pSA13, but in opposite orientation	This study
pSA13Δ1070	pSA13 with 1,070-bp *Bss*HII fragment deleted	This study
pSA13Δ616	pSA13 with 616-bp *Bss*HII fragment deleted	This study
pSA13Δ454	pSA13 with 454-bp *Bss*HII fragment deleted	This study

ternational Biotechnologies Inc. Polynucleotide kinase was obtained from New England BioLabs. DNA polymerase I-DNase I mixture was obtained from Bethesda Research laboratories, and calf intestinal alkaline phosphatase was purchased from Boehringer Mannheim Biochemicals. [α-^{32}P]deoxynucleoside triphosphates and [γ-^{32}P]ATP were obtained from Du Pont-New England Nuclear. Acrylamide, bisacrylamide, and ultrapure urea were obtained from Schwarz-Mann Biotech. Other reagents were purchased from Sigma Chemical Co.

Construction of subclones for sequencing. A 1.4-kilobase-pair (kbp) *Eco*RI-*Eco*RV fragment from pMC4 containing the *hutC* region was isolated by agarose gel electrophoresis and phenol-freeze extraction (2) and partially digested with *Sau*3AI to produce a mixture of overlapping fragments. This mixture was used in the ligation reaction with the plasmid vector pUC8 which had been cut with *Bam*HI, producing compatible ends for ligation. This resulted in the construction of several clones with inserts of different sizes and the same polylinker restriction sites flanking the insert in each clone. The plasmids pSA1, pSA5, pSA7, and pSA12 each contained inserts derived from pMC4 (Table 1). *E. coli* RDP210 (pSA1), RDP145 (pSA5 and pSA7), pUC8 (pSA12 and other pUC8 constructions), and N4830 (pPL-lambda derivatives) were transformed by the $CaCl_2$-RbCl method (17).

Plasmid preparation and DNA sequencing. Plasmids were isolated from 2-liter cultures grown at 30°C in LB broth containing ampicillin (50 μg/ml) and amplified overnight with chloramphenicol (170 μg/ml) as described before (17). Cells were harvested and washed, and plasmids were obtained by the alkaline lysis method (17) and purified by CsCl equilibrium density gradient centrifugation in a Beckman L8-70M ultracentrifuge at 45,000 rpm for 36 h at 20°C with an 80Ti rotor.

Plasmids were digested with appropriate restriction enzymes and labeled at the 3′ end with Klenow fragment and the appropriate [α-^{32}P]deoxynucleoside triphosphate or at the 5′ end with calf intestinal alkaline phosphatase, polynucleotide kinase, and [γ-^{32}P]ATP. DNA fragments were separated by electrophoresis in ultrapure agarose and isolated by the phenol-freeze technique of Benson (2).

DNA sequencing was done by the chemical cleavage method of Maxam and Gilbert (18). When pUC8-derived constructions were used, the *Sal*I, *Ava*I, *Hin*dIII, and *Eco*RI sites of the polylinker region were used to cut out and label the insert DNA. In some cases, fragments from pSA1, pSA5, PSA7, and pSA12 were used to complete the sequence of both strands and to confirm the ordering of fragments at the *Sau*3AI sites.

Repressor assay. Repressor assays were carried out by the filter technique described previously by Johnson et al. (14) with the modifications of Hu et al. (11). Cultures were grown at 30°C in L-broth to 200 Klett units and shifted to 42°C. After 2 h the cells were harvested by centrifugation and suspended in 0.5 ml of TEDG buffer (10 mM Tris hydrochloride [pH 7.5], 0.1 mM EDTA, 0.1 mM dithiothreitol, 100 mM NaCl, and 10% glycerol). Crude extracts were prepared by sonic treatment as described before (11). Protein was determined by the method of Bradford (5) with ovalbumin as a standard. Various DNA fragments were isolated from pMC4, labeled by nick translation (17), and assayed for DNA-binding ability by using various amounts of repressor in the presence or absence of 5 mM urocanate.

RESULTS

Nucleotide sequence of the *hutC* region. Several subclones of the plasmid pMC4, which contains a functional Hut repressor gene, were constructed in order to facilitate sequencing of the *hutC* locus. Figure 1 shows the relationship of these subclones to the parent plasmid. The organization of the *hut* genes was described previously (13).

The nucleotide sequence of the *hutC* region was determined in both strands and is shown in Fig. 2. The sequence revealed two open reading frames. The first open reading frame, designated ORF1, was 744 bases long and predicted to encode a polypeptide with a molecular weight of 27,648. It began with a GTG initiation codon and was preceded by the sequence AAGGA, which is expected to serve as a binding

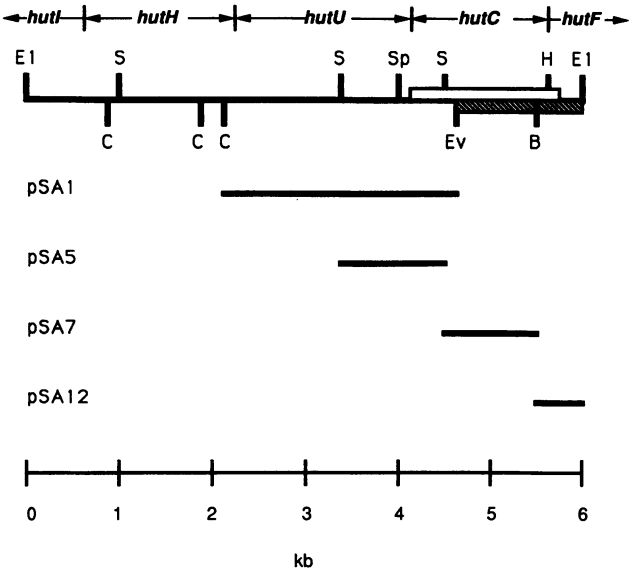

FIG. 1. Subclones used in sequencing of the *hutC* region. The figure shows a partial restriction map of the pMC4 insert and the relative positions of the *hutC*, *hutU*, and *hutH* genes. The restriction fragments used in construction of subclones of pMC4 are shown. E1, *Eco*RI; C, *Cla*I; S, *Sal*I; Sp, *Ssp*I; Ev, *Eco*RV; B, *Bam*HI; H, *Hpa*I. The hatched box shows the *Eco*RI-*Eco*RV fragment used to generate the random *Sau*3AI fragments that were cloned into pUC8. The open box corresponds to the sequenced region shown in Fig. 2.

site for the *Pseudomonas* 30S ribosome (9). The second open reading frame (ORF2) was 570 bases long and had the capacity to encode a polypeptide with a molecular weight of 21,155. The ATG initiation codon of ORF2 overlapped the TGA termination codon of ORF1, and they were in different reading frames. ORF2 also had the sequence AAGGA preceding it, indicating that it has the potential to be translated.

Both ORF1 and ORF2 showed a biased codon usage, with 79.4% (197 of 248) of the codons in ORF1 and 77.9% (148 of 190) of the codons in ORF2 ending in either G or C. This bias is consistent with what has been observed for other *Pseudomonas* coding regions (30) and further suggests that both open reading frames may encode protein products.

The sequences TATACA and TAAACT, approximately 70 and 45 bp, respectively, upstream from the beginning of ORF1, resembled the common −35 and −10 consensus sequence for bacterial promoters (25) and constituted a likely promoter site for the *hutC* transcript. Adjacent to this putative promoter region was the sequence CTTGTATATA CATA, which is nearly identical to the sequence CTTGTA CATACAAG that was shown previously to be part of the recognition site for binding of the Hut repressor protein (11). It appears that RNA polymerase and Hut repressor would compete for binding in this region.

The region extending from nucleotides 1516 to 1543 (Fig. 2) was a G+C-rich symmetrical sequence which should be capable of forming a strong hairpin structure. Analysis of this region was performed on a VAX 3600 computer with the Terminator program (University of Wisconsin Genetics Computer Group). By the algorithm of Brendel and Trifonov (6, 7), a likely transcriptional termination site was found with a $p-$ (primary structure) value of 3.56 and an $s-$ (secondary structure) value of 83. The presence of a transcriptional terminator at this site would be consistent with earlier evidence of independent transcription units for *hutC* and *hutUHIG* (13).

Comparison with other *hut* regulatory proteins. Neither ORF1 nor ORF2 showed significant sequence homology to the positive regulatory gene of the *Bacillus subtilis hut* operon (20). However, the predicted amino acid sequence of the ORF1 protein showed 62% identity to the predicted *hutC* sequence of *Klebsiella aerogenes* (27). The two sequences showed several regions of strong sequence conservation (Fig. 3), including a portion of the putative DNA recognition region (Fig. 4).

Comparison with other DNA-binding proteins. The region of ORF1 extending from nucleotide 301 to nucleotide 360 (Fig. 2) is predicted to encode an amino acid sequence resembling the helix-turn-helix motif common to DNA-binding proteins (21). Although a FASTP search of the Protein Identification Resource data base did not reveal any strongly related sequences, alignment of the putative DNA-binding domain of ORF1 with the DNA-binding regions of *Escherichia coli* Trp repressor and CAP proteins (Fig. 4) showed considerable amino acid sequence similarity in this region. Chou-Fasman analysis (Protein Identification Resource) confirmed that this region, containing amino acids 46 to 65, lay within a probable α-helix-forming region. Direct evidence that this portion of the Hut repressor is responsible for binding of DNA at some or all of the *hut* operator regions has not yet been obtained.

Construction of an expression plasmid containing ORF1 and ORF2. The two open reading frames identified in the DNA sequence were contained within a 1,549-bp *Hpa*I-*Ssp*I fragment. The plasmids pSA13 and pSA14 were constructed by inserting this fragment in both orientations into the *Hpa*I cloning site of pPL-lambda (Fig. 5). These plasmids were then used to transform *E. coli* N4830, which encodes a temperature-sensitive lambda repressor that allows expression from the p_L promoter when the temperature is raised from 28 to 42°C (26).

The ability of crude extracts of N4830(pSA13) and N4830(pSA14) to bind the *hutUHIG* operator site was tested by using the nitrocellulose filter assay (11). As shown in Fig. 6, neither N4830(pSA13) nor N4830(pSA14) showed any binding activity when cells were grown at 28°C. When cells were shifted to 42°C for 2 h, N4830 (pSA13) showed binding activity, but N4830(pSA14) did not. Addition of 5 mM urocanate to the assay mixture reduced the binding. These results, together with earlier genetic evidence (8, 12, 13), confirm that the sequenced region contains the gene for the *hut* repressor and that the gene is oriented in the predicted direction.

When a 2.9-kb *Xho*I fragment containing the *hutG* operator (11) was used as a probe to measure binding to this operator, specific binding activity was not observed in the crude extracts from either N4830(pSA13) or N4830(pSA14) (data not shown). This may be attributable to the previously observed lower affinity of binding to the *hutG* operator than to the *hutUHIG* operator (11), causing the signal to be obscured by other nonspecific interactions in the crude extracts.

Deletion of ORF2 region. The plasmid pSA13 contains three *Bss*HII sites within the insert DNA. The first is approximately in the middle of ORF1, the second is 17 bp downstream from the end of ORF1, and the third is 64 bp downstream from the end of ORF1. Three different deletion subclones were constructed by using these *Bss*HII sites (Fig. 5). Deletion of the entire region from the first to the third site resulted in a 1,070-bp deletion in which only the N-terminal

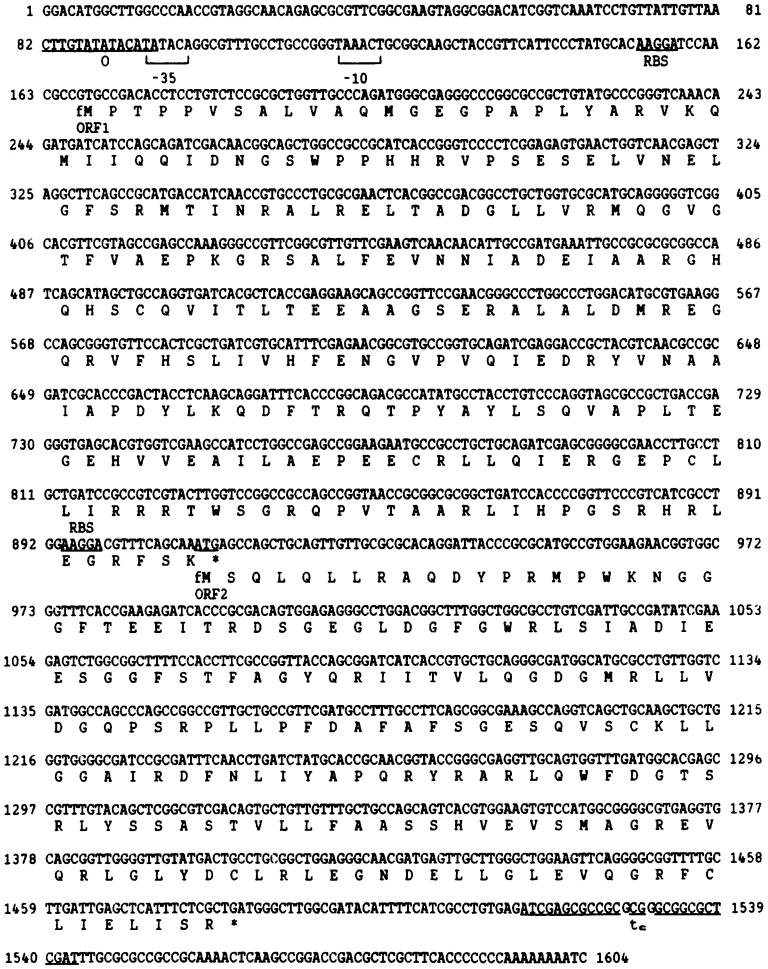

FIG. 2. Nucleotide sequence and predicted amino acid sequence of the *hutC* region (GenBank, EMBL, and DDBJ accession no. M33922). The portion of the DNA sequence containing ORF1 and ORF2 is shown. The putative promoter sequences are underlined and labeled −35 and −10. The underlined region indicated by O is the probable repressor recognition sequence. Potential ribosome-binding sites are marked (RBS). The putative transcriptional terminator site is labeled t_c, and the start codon of ORF2 that overlaps the stop codon of ORF1 is underlined. Symbols for amino acids are aligned with the second base of each codon. FM, *N*-Formylmethionine.

portion of ORF1 and none of ORF2 was present. Deletion of the 454-bp region between the first and second *Bss*HII sites left the N-terminal portion of ORF1 intact, and although most of ORF2 was present in this construction, the N-terminal end of ORF2 was deleted, so that translation of ORF2 would not be expected to occur in this construction. Deletion of the 616 bp between the second and third *Bss*HII sites left all of ORF1 intact but eliminated all but 17 bp of ORF2.

FIG. 3. Comparison of *P. putida* ORF1 and *K. aerogenes hut* repressor sequences. The predicted amino acid sequences of the ORF1 protein of *P. putida* (line 1) and the *K. aerogenes hut* repressor (27) (line 2) are shown. Exact matches are indicated by vertical lines.

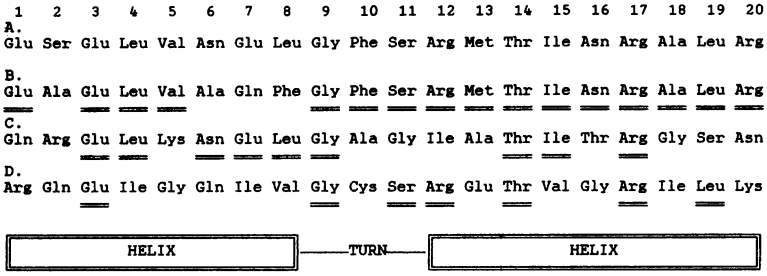

FIG. 4. Comparison of *P. putida hut* repressor with selected DNA-binding proteins. The amino acid sequence of residues 46 to 65 of the predicted ORF1 protein was aligned to show similarity to the helix-turn-helix binding domain commonly found in procaryotic DNA-binding proteins (21). (A) *P. putida hut* repressor; (B) *K. aerogenes hut* repressor; (C) *E. coli* Trp repressor; (D) *E. coli* CAP protein. Double-underlined residues represent identity with the *P. putida hut* repressor sequence.

When a probe containing the *hutUHIG* operator was used to carry out filter assays on crude extracts of temperature-induced cells, the 1,070- and 454-bp deletions resulted in the loss of observable repressor activity, but the 616-bp deletion, in which ORF2 was selectively removed, did not cause in vitro binding activity to be lost (Fig. 6). It therefore appears that ORF2 is not essential for *hut* repressor activity at the *hutUHIG* operator site.

DISCUSSION

The nucleotide sequence of the *hutC* region revealed the existence of two open reading frames capable of encoding polypeptides with molecular weights of approximately 27,000 and 21,000. The product of the first open reading frame appears to contain a potential helix-turn-helix motif, which is characteristic of procaryotic DNA-binding proteins. We predict that this region will prove to be important for the recognition and binding of *hut*-specific operator sites. Hu et al. (11, 12) have proposed that the interaction between repressor and the operator region preceding the *hutG* gene might be different from those involving the *hutUHIG* and *hutF* operators. It is possible that the putative DNA-binding domain that we have identified in the ORF1 protein is necessary for binding one type of operator sequence while a separate protein domain is involved in the other interaction.

The predicted ORF1 protein shows a considerable sequence similarity to the *K. aerogenes hutC* protein (27). Of particular interest are several regions with a high degree of sequence conservation, including a portion of the putative DNA-binding region. The conserved region includes the turn residues and the second helix, which, by analogy to other DNA-binding proteins, would be expected to interact with base pairs of the major groove of the operator DNA and serve as a recognition domain for the operator sequence (21).

Although the role of ORF2 is less certain, it appears that it would be included in the same transcriptional unit as ORF1, allowing the synthesis of these two polypeptides to be coordinated. One possible role of ORF2 might be to encode a second subunit of a heteromeric repressor protein composed of the ORF1 and ORF2 gene products. However, the fact that this region can be deleted entirely with little effect on binding at the *hutUHIG* operator site makes this unlikely. Nevertheless, further analysis with purified protein may reveal an involvement of ORF2 with the binding at the *hutG* operator which could not be observed in the filter assay with crude extracts. Alternatively, the ORF2 protein might be a separate regulatory factor that somehow modifies or enhances the effect of the *hut* repressor. Either of these possibilities, if found to be true, would have interesting implications pertaining to the proposed bifunctional nature of the *hut* repressor (12).

The observation that the 5'-flanking region preceding

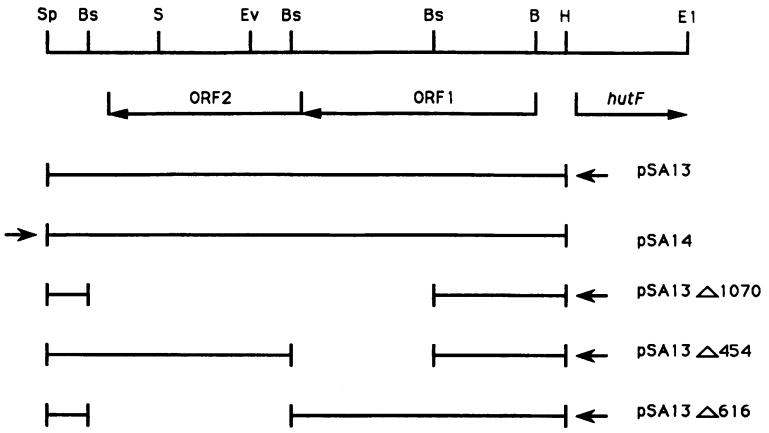

FIG. 5. Subclones for expression of *hut* repressor. An *Hpa*I-*Ssp*I fragment containing all of ORF1 and ORF2 was cloned in both orientations into pPL-lambda, resulting in plasmids pSA13 and pSA14. The location of this fragment is shown relative to the restriction map. The plasmids pSA13 Δ1070, pSA13Δ454, and pSA13Δ616 were constructed by excising various *Bss*HII fragments from pSA13 and religating. The gaps indicate the portions that were deleted in each construction. The orientation of the p_L promoter in each clone is represented by a short arrow. See Fig. 1 legend for abbreviations.

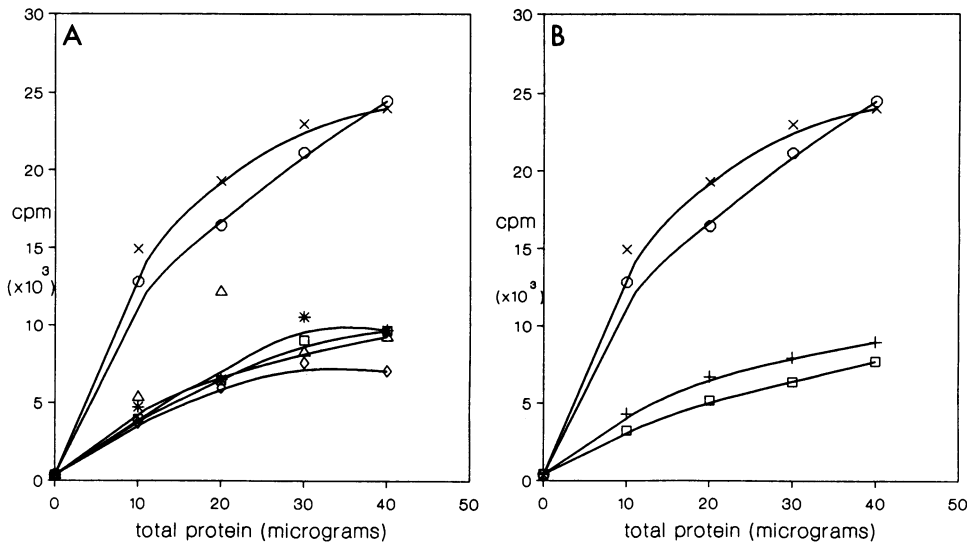

FIG. 6. DNA-binding activities of pSA13, pSA14, and deletion subclones. (A) Filter-binding analysis of repressor activity in extracts from *E. coli* N4830 containing the various plasmid constructions shown in Fig. 5. Extracts were prepared from cells that were grown to 200 Klett units at 28°C and shifted for 2 h to 42°C to induce expression from the p_L promoter. Symbols: ○, pSA13; △, pSA14; *, pSA13Δ1070; □, pSA13Δ454; ×, pSA13Δ616; ◇, host strain without plasmid. (B) Effect of inducer on operator binding. Assays were done in the presence or absence of urocanate. Symbols: ○, pSA13; ×, pSA13Δ616; □, pSA13 plus 5 mM urocanate; +, pSA13Δ616 plus 5 mM urocanate.

ORF1 contains a potential repressor-binding site in the putative promoter region strongly suggests that the *hut* repressor regulates its own synthesis by competing with RNA polymerase binding at the promoter site. We have recently found (unpublished data) that the operator site and the promoter sites are functional and that urocanate abolishes binding at the operator site in vitro. This would suggest that under inducing conditions (high urocanate concentration), the repressor protein would continue to be synthesized and would be available for rapid shutdown of Hut enzyme synthesis when the source of histidine (and urocanate) is depleted.

Earlier work (13) has shown that the *hutF* gene maps close to, and is oriented in the opposite direction from the *hutC* gene. It is also known that expression of *hutF* is controlled by the *hutC* gene product and is inducible by urocanate (8, 13, 16). We have preliminary evidence that the promoter site for the *hutF* transcript is located in this region. It can therefore be speculated that a single repressor-binding site might serve to function as a regulatory locus for both the *hutC* and *hutF* transcripts. Several other examples of regulation of divergent promoters by a single regulatory protein have already been encountered (1).

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LITERATURE CITED

1. **Beck, C. F., and R. A. J. Warren.** 1988. Divergent promoters, a common form of gene organization. Microbiol. Rev. **52**:318–326.
2. **Benson, S. A.** 1984. A rapid procedure for isolation of DNA fragments from agarose gels. Biotechniques **2**:66–67.
3. **Bolivar, F., R. L. Rodriguez, P. J. Greene, M. C. Betlach, H. L. Heyneker, H. W. Boyer, J. H. Crosa, and S. Falkow.** 1977. Construction and characterization of new cloning vehicles. II. A multipurpose cloning system. Gene **2**:95–113.
4. **Boylan, S. A., and R. A. Bender.** 1984. Genetic and physical maps of *Klebsiella aerogenes* genes for histidine utilization (*hut*). Mol. Gen. Genet. **193**:99–103.
5. **Bradford, M. M.** 1976. A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. Anal. Biochem. **72**:248–254.
6. **Brendel, V., and E. N. Trifonov.** 1984. A computer algorithm for testing potential prokaryotic terminators. Nucleic Acids Res. **12**:4411–4427.
7. **Brendel, V., and E. N. Trifonov.** 1985. Computer-aided mapping of DNA-protein interaction sites, p. 115–118. *In* P. S. Glaeser (ed.). The role of data in scientific progress. Elsevier/North-Holland, Amsterdam.
8. **Consevage, M. W., R. D. Porter, and A. T. Phillips.** 1985. Cloning and expression in *Escherichia coli* of histidine utilization genes from *Pseudomonas putida*. J. Bacteriol. **162**:138–146.
9. **Frantz, B., and A. M. Chakrabarty.** 1986. Degradative plasmids in *Pseudomonas*, p. 295–323. *In* J. R. Sokatch (ed.), The bacteria, vol. X: the biology of *Pseudomonas*. Academic Press, Inc., London.
10. **Goldberg, R. B., and B. Magasanik.** 1975. Gene order of the histidine utilization (*hut*) operons in *Klebsiella aerogenes*. J. Bacteriol. **120**:1025–1031.
11. **Hu, L., S. L. Allison, and A. T. Phillips.** 1989. Identification of multiple repressor recognition sites in the *hut* system of *Pseudomonas putida*. J. Bacteriol. **171**:4189–4195.
12. **Hu, L., L. M. Mulfinger, and A. T. Phillips.** 1987. Purification and properties of formylglutamate amidohydrolase from *Pseudomonas putida*. J. Bacteriol. **169**:4696–4702.
13. **Hu, L., and A. T. Phillips.** 1988. Organization and multiple regulation of histidine utilization genes in *Pseudomonas putida*. J. Bacteriol. **170**:4272–4279.
14. **Johnson, A. D., C. O. Pabo, and R. T. Sauer.** 1981. Bacteriophage lambda repressor and Cro protein: interactions with operator DNA. Methods Enzymol. **65**:839–865.
15. **Kimhi, Y., and B. Magasanik.** 1970. Genetic basis of histidine degradation in *Bacillus subtilis*. J. Biol. Chem. **245**:3545–3548.
16. **Leidigh, B. J., and M. L. Wheelis.** 1973. Genetic control of the histidine dissimilatory pathway in *Pseudomonas putida*. Mol. Gen. Genet. **120**:201–210.
17. **Maniatis, T., E. F. Fritsch, and J. Sambrook.** 1982. Molecular cloning: a laboratory manual. Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y.

18. **Maxam, A. M., and W. Gilbert.** 1980. Sequencing end-labeled DNA with base-specific chemical cleavage. Methods Enzymol. **65:**499–560.
19. **Nieuwkoop, A. J., S. A. Boylan, and R. A. Bender.** 1984. Regulation of *hutUH* operon expression by the catabolite gene activator protein-cyclic AMP complex in *Klebsiella aerogenes*. J. Bacteriol. **159:**934–939.
20. **Oda, M., A. Sugishita, and K. Furukawa.** 1988. Cloning and nucleotide sequences of histidase and regulatory genes in the *Bacillus subtilis hut* operon and positive regulation of the operon. J. Bacteriol. **170:**3199–3205.
21. **Pabo, C. O., and R. T. Sauer.** 1984. Protein-DNA recognition. Annu. Rev. Biochem. **53:**293–321.
22. **Phillips, A. T., and L. M. Mulfinger.** 1981. Cyclic adenosine 3′, 5′-monophosphate levels in *Pseudomonas putida* and *Pseudomonas aeruginosa* during induction and carbon catabolite repression of histidase synthesis. J. Bacteriol. **145:**1286–1292.
23. **Potts, J. R., and P. H. Clarke.** 1976. The effect of nitrogen limitation on catabolite repression of amidase, histidase and urocanase in *Pseudomonas aeruginosa*. J. Gen. Microbiol. **93:**377–387.
24. **Prival, M. J., and B. Magasanik.** 1971. Resistance to catabolite repression of histidase and proline oxidase during nitrogen-limited growth of *Klebsiella aerogenes*. J. Biol. Chem. **246:** 6288–6296.
25. **Rosenberg, M., and D. Court.** 1979. Regulatory sequences involved in the promotion and termination of RNA transcription. Annu. Rev. Genet. **13:**319–353.
26. **Rosenberg, M., Y.-S. Ho, and A. Shatzman.** 1983. The use of pKC30 and its derivatives for controlled expression of genes. Methods Enzymol. **101:**123–138.
27. **Schwacha, A., and R. A. Bender.** 1990. Nucleotide sequence of the gene encoding the repressor for the histidine utilization genes of *Klebsiella aerogenes*. J. Bacteriol. **172:**5477–5481.
28. **Smith, G. R., and B. Magasanik.** 1971. The two operons of the histidine utilization system in *Salmonella typhimurium*. J. Biol. Chem. 246:3330–3341.
29. **Vieira, J., and J. Messing.** 1982. The pUC plasmids, an M13mp7-derived system for insertion mutagenesis and sequencing with synthetic universal primers. Gene **19:**259–268.
30. **West, S. E. H., and B. H. Iglewski.** 1988. Codon usage in *Pseudomonas aeruginosa*. Nucleic Acids Res. **16:**9323–9335.