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Nucleotide Sequence of the *uhp* Region of *Escherichia coli*

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The *Escherichia coli uhp* region encodes the transport system that mediates the uptake of a number of sugar phosphates as well as the regulatory components that are responsible for induction of this transport system by external glucose 6-phosphate. Four *uhp* genes have been identified by analysis of the complementation behavior and polypeptide coding capacity of plasmids carrying subcloned regions or transposon insertions. The nucleotide sequence of a 6.5-kilobase segment that contains the 3′ end of the *ilvBN* operon and the entire *uhp* region was determined. Four open reading frames were identified in the locations expected for the various *uhp* genes; all were oriented in the same direction, counterclockwise relative to the genetic map. The properties of the polypeptides predicted from the nucleotide sequence were consistent with their observed features. The 196-amino-acid UhpA polypeptide has the composition characteristic of a soluble protein and bears homology to the DNA-binding regions of many regulatory activators and repressors. The 518-amino-acid UhpB and the 199-amino-acid UhpC regulatory proteins contain substantial segments of hydrophobic character. Similarly, the 463-amino-acid UhpT transporter is a hydrophobic protein with numerous potential transmembrane segments. The UhpC regulatory protein has substantial sequence homology to part of UhpT, suggesting that this regulatory protein might have evolved by duplication of the gene for the transporter and that its role in transmembrane signaling may involve sugar-phosphate-binding sites and transmembrane orientations similar to those of the transport protein.

Expression of the *Escherichia coli* sugar phosphate transport system is induced by external glucose 6-phosphate, but is unaffected by glucose 6-phosphate generated internally from phosphorylation of exogenous glucose (33). Previous studies using *uhpT-lac* operon fusions showed that induction of the transporter gene *uhpT* occurred normally even in strains lacking detectable glucose 6-phosphate uptake activity (25). The characteristics of Uhp expression suggested that regulation involves transmembrane signaling, in which a membrane-bound regulatory protein serves as the receptor for external glucose 6-phosphate and triggers events that result in elevated transcription of the *uhpT* gene (32).

All genes specifically involved in the production of the Uhp transport system are linked in the *uhp* region at min 82.1 of the *E. coli* genetic map (12, 13). The accompanying paper describes the location of the four *uhp* genes and their requirement for Uhp expression (32). Of the three regulatory genes, *uhpA* appears to encode a positive activator of *uhpT* transcription, as suggested by its absolute requirement and the constitutive expression of the UhpT transporter when *uhpA* is present at elevated gene dosage. Two other genes, *uhpB* and *uhpC*, encode proteins that are required for *uhpT* expression except when *uhpA* is present in multicopy plasmids. Complementation results also suggested that the UhpT transporter is comprised of only a single polypeptide.

This paper communicates the nucleotide sequence of the *uhp* region carried on a 6.5-kilobase (kb) *Hin*dIII-*Eco*RV fragment from the Clarke-Carbon plasmid pLC17-47 (26). The location of the open reading frames correlated well with the location of the genes defined by subclones and transposon insertions (32). Homologies of portions of the polypeptides deduced from the nucleotide sequence support a model for regulation through transmembrane activation of a transcriptional activator.

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MATERIALS AND METHODS

Determination of nucleotide sequence. DNA sequencing was performed by the dideoxy chain termination method of Sanger et al. (23), using [α-^{35}S]thio-dCTP (5). Single-stranded DNA templates were derivatives of the phage vectors M13 mp18 and M13 mp19 (18) carrying restriction fragments from the *uhp* region of plasmid pRJK10 (32). These included small restriction fragments generated by cleavage with *Sau*3A, *Taq*I, *Hpa*II, or *Pvu*II. Larger fragments were generated by isolation and ligation of the 1.58-kb *Hpa*I, the 1.96-kb *Cla*I, the 4.2-kb *Cla*I-2–*Bam*HI, the 2.4-kb *Sph*I-*Bam*HI, the 790-base-pair *Sph*I-*Bgl*II, and the four *Eco*RV fragments in both orientations in the vectors cut with appropriate restriction enzymes.

Nested deletions extending into the larger DNA fragments from the end proximal to the universal primer-binding site on the vector were generated as described by Dale et al. (7). In this method, single-stranded viral DNA is converted to linear form by cleavage with an appropriate restriction enzyme (*Hin*dIII or *Eco*RI) after hybridization to the template of an oligonucleotide complementary to the sequences around the restriction site. Treatment of the linear DNA with T4 DNA polymerase results in generation of deletions into the insert by means of the enzyme's 3′ to 5′ exonuclease activity. Recircularization is then accomplished by addition of a short 3′ homopolymer tail, followed by annealing to an oligonucleotide complementary to both ends and subsequent ligation of the juxtaposed ends.

Gel readings of areas of high G+C content often resulted in sequence anomalies (compression). More reliable readings through these areas were obtained by substitution of 7-deazadeoxyguanosine 5′-triphosphate in place of dGTP (3).

The management of sequence information and subsequent analysis of compiled data was effected by the DBUTIL programs of Staden (29). Hydropathy profiles were deter-

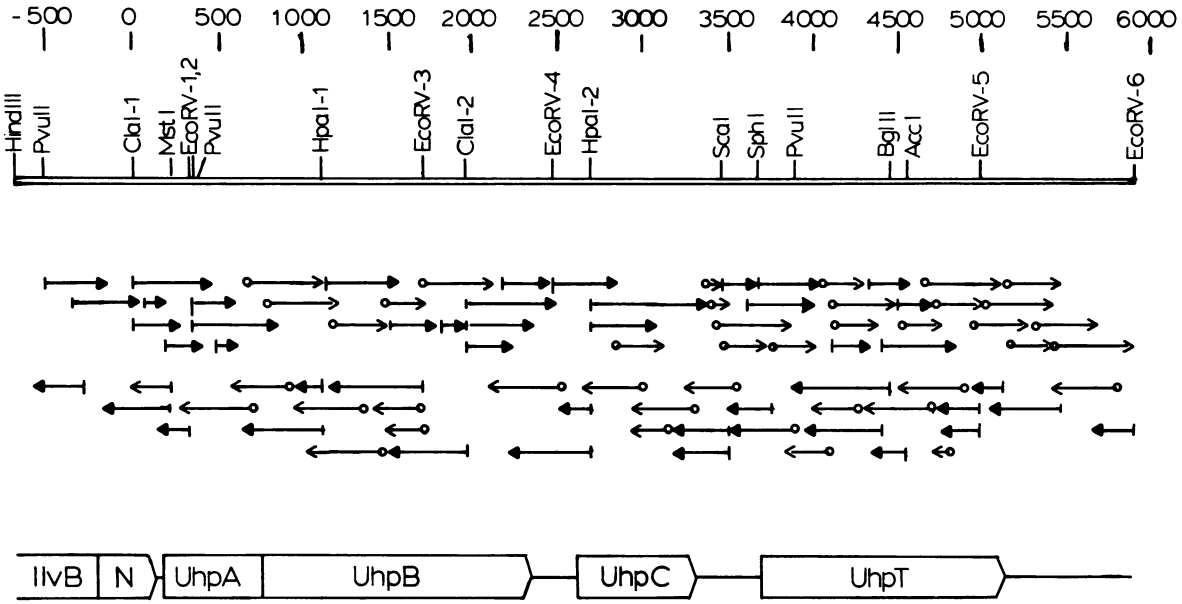


FIG. 1. Restriction map of the *uhp* region and sequencing strategy. The top line presents the location of restriction enzyme cleavage sites that were used for determination of the nucleotide sequence or for construction of subcloned plasmids. Multiple sites for a single enzyme are numbered from left to right. Numbering begins at the *Cla*I-1 site. The sequencing strategy diagrammed in the central section gives the length and direction of each fragment whose sequence was determined. Lines with filled arrowheads and beginning with a vertical line represent restriction fragments, whereas lines with open arrowheads with a circle represent deletions generated by the action of T4 DNA polymerase. At the bottom is presented schematically the location of the open reading frames and their gene assignments, as described in the text.

mined by using the parameters of Kyte and Doolittle (15) or Engelman et al. (8). Comparisons of amino acid sequences used the FASTP program of Lipman and Pearson (16).

Reagents. Restriction endonucleases and T4 DNA ligase were purchased from New England Biolabs, Inc., or Boehringer-Mannheim Biochemicals and were employed under the conditions recommended by their manufacturers. The DNA sequencing kits and [α-^{35}S]thio-dCTP were obtained from Amersham Corp. The T4 DNA polymerase and oligonucleotides used for generation of deletions were obtained from International Biotechnologies, Inc.

RESULTS AND DISCUSSION

Nucleotide sequence of the *uhp* region. The nucleotide sequence of the 6.5-kb *Hin*dIII–*Eco*RV-6 DNA fragment carried in plasmid pRJK10 was determined by means of the sequencing strategy shown in Fig. 1. Both defined restriction fragments and random deletions generated by the action of T4 DNA polymerase on single-stranded phage M13 templates were analyzed. Numbering of the nucleotides begins at the *Cla*I-1 site. Except for a 124-base-pair stretch (nucleotides 1956 to 2080), sequence data were obtained for both strands, with overlaps covering all restriction sites. The assignment of each nucleotide was based on an average of 6.7 gel readings.

Identification of open reading frames in the *uhp* region. The genetic studies reported in the accompanying paper indicated the existence of four *uhp* genes (32). Four translational reading frames of appropriate sizes were deduced from the nucleotide sequence (Fig. 2), in locations consistent with the *uhp* gene boundaries defined by transposon insertions and subclones. The coding region of the *ilvBN* operon ends at nucleotide 84 and is followed by a typical Rho-independent transcription termination sequence, as described by Wek et al. (31) and Friden et al. (9).

Just past this termination sequence, the putative *uhpA* reading frame extends from nucleotide 163 to nucleotide 750, encoding a 196-amino-acid polypeptide of M_r 20,893. A potential Shine-Dalgarno ribosome-binding sequence (AGGA) (28) is located 7 nucleotides upstream from the start of this reading frame. No other potential initiation site in this or another reading frame (at nucleotides 302, 304, 310, 312, 331, 379, 391, or 479) was preceded by this collection of characteristic expression signals, although amino-terminal sequence information is necessary to define the actual start site.

The *uhpA* gene ends with the sequence TGATGA at nucleotide 751; the ATG in this sequence is at the beginning of the long *uhpB* reading frame between nucleotides 753 and 2306. This reading frame is preceded by a possible Shine-Dalgarno sequence (GATGG) and encodes a 518-amino-acid polypeptide of M_r 58,642. Other possible initiation sites for this gene (nucleotides 864, 870, or 906) are not preceded by likely Shine-Dalgarno sites. It is possible that the translation of *uhpA* and *uhpB* is coupled, although there is no direct evidence for this point. The location of the 3′ end of the *uhpB* reading frame is in agreement with the end of the gene defined by transposon insertions. Insertion 4 in *uhpB* is located between nucleotides 1956 (the *Cla*I-2 site) and 2341 (an *Aha*II site), whereas insertion 28, which lies outside *uhpB*, is located between nucleotides 2341 and 2459 (the *Eco*RV-4) site (32).

A third open reading frame is found between nucleotides 2457 and 3272. However, the first initiation codon is the ATG at nucleotide 2616, which would predict a 219-amino-acid polypeptide product with M_r 23,790. This initiation codon is preceded by a very poor ribosome-binding sequence (GA), and the predicted polypeptide is substantially larger than the observed M_r 20,000 UhpC product. It is more likely that the *uhpC* coding sequence is initiated at the GTG codon at nucleotide 2676. Translation from this site would

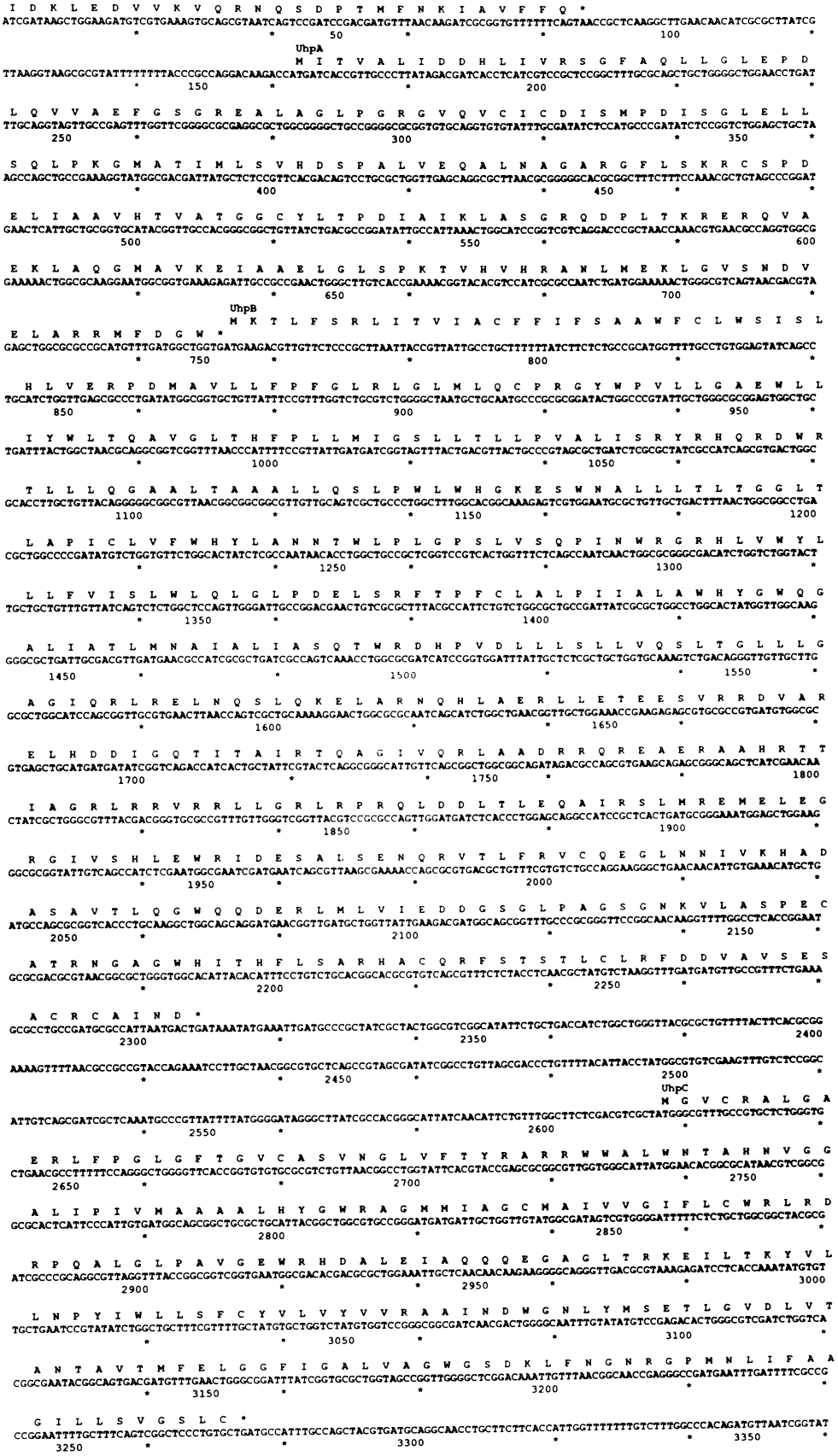
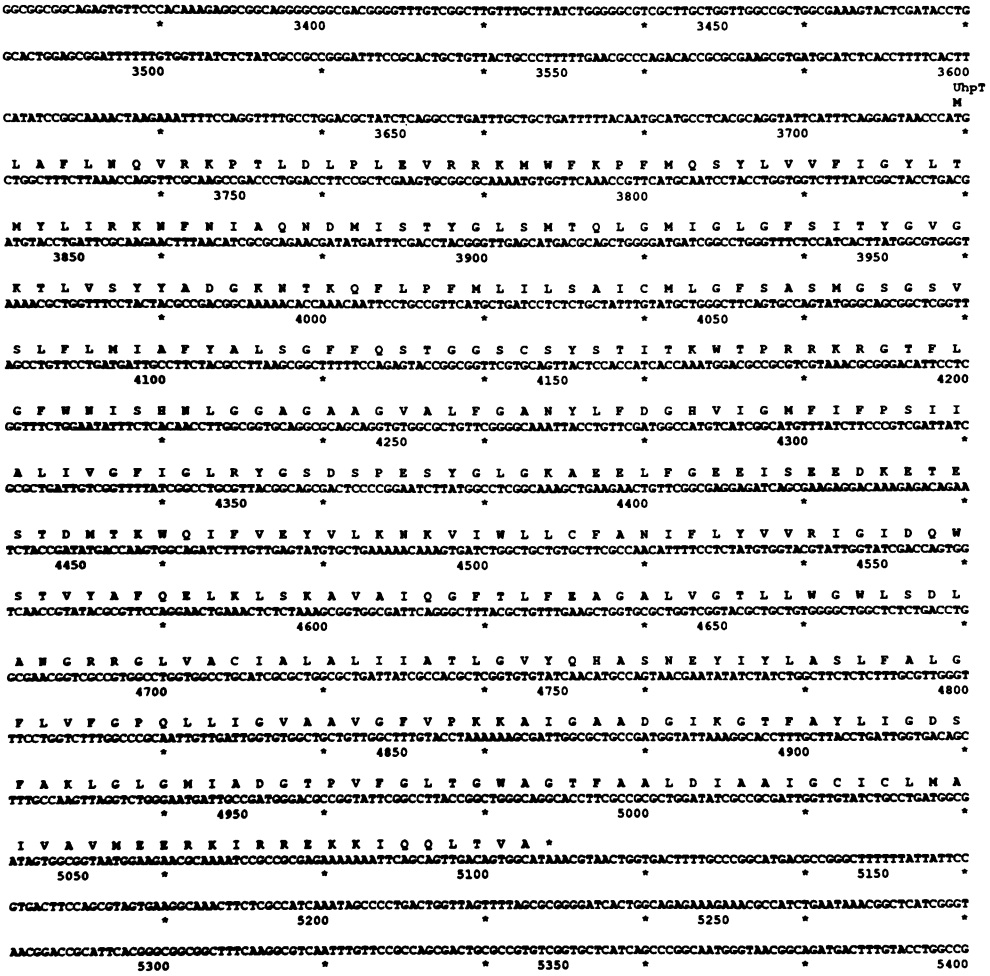


FIG. 2. Nucleotide sequence of the *uhp* region. The nucleotide sequence extending for 5,400 base pairs from the *Cla*I-1 site is presented. The predicted amino acid sequences for the *uhp* open reading frames are indicated in one-letter code.



generate a 199-amino-acid polypeptide with a molecular weight of 21,787. Upstream of this start site is a polypurine stretch, GGGG, which could serve as a ribosome-binding sequence. Transposon insertion 33 in *uhpC* is located between nucleotides 2462 (*Eco*RV-3) and 2690 (*Hpa*I-2), consistent with the identification of this reading frame as *uhpC*. The location of the actual start site must await analysis of the amino-terminal polypeptide sequence.

The reading frame for the *uhpT* gene extends between nucleotides 3718 and 5106 and encodes a 463-amino-acid polypeptide of M_r 50,615. All of the transposon insertions that specifically inactivate *uhpT* are located within this region. A Shine-Dalgarno sequence (AGGAG) is centered 8 nucleotides upstream from the coding region. The termination codon is followed by a typical Rho-independent transcription terminator structure, namely, a 7-base-pair GC-containing stem with a 5-nucleotide loop (ΔG = −21.9 kcal/mol [ca. −91.6 kJ/mol]) (22), and followed by six contiguous T residues (21).

Identification of potential transcriptional regulatory signals. There is a potential promoter sequence located between nucleotides 99 and 137, just upstream from the *uhpA* coding region. This sequence contains a −35 TTGAAC and a −10 TAAGGT region separated by 17 nucleotides; the invariant T residue in the −10 region is located 7 nucleotides upstream from a potential transcription initiation site (TAT) (21). The poor match of this putative promoter sequence with the canonical sequence is consistent with the relatively low level of expression of the *uhp* regulatory genes (unpublished data). This promoter overlaps the *ilvBN* terminator such that the run of T residues in the terminator would be the first nucleotides in the *uhpA* transcript.

Since transposon insertions in *uhpA* have a polar effect on expression of *uhpB* (32), it is likely that both genes are transcribed from the same promoter into a polycistronic message. No promoter sequences were detected in front of *uhpB*, but a weak promoter might have been missed. The poor expression of *uhpC* in maxicells makes it uncertain whether this gene is part of the same operon as the other regulatory genes. Transposon insertion 28, lying between *uhpB* and *uhpC*, does not confer a Uhp$^-$ phenotype, suggesting that the insertion does not eliminate expression of *uhpC*. However, these results do not preclude a reduction in synthesis of this protein. Potential weak promoter sequences can be found upstream of *uhpC* (for example, between nucleotides 2542 and 2556), but determination of their significance will require transcript mapping.

A potential promoter region is seen in front of *uhpT*. A transcription initiation sequence CAC (nucleotides 3689 to 3691) is located 17 nucleotides before the Shine-Dalgarno sequence. This start site is preceded by a typical −10 sequence TACAATG 7 nucleotides upstream. There is, however, no sequence resembling the consensus −35 sequence at the expected location. Instead, the symmetrical

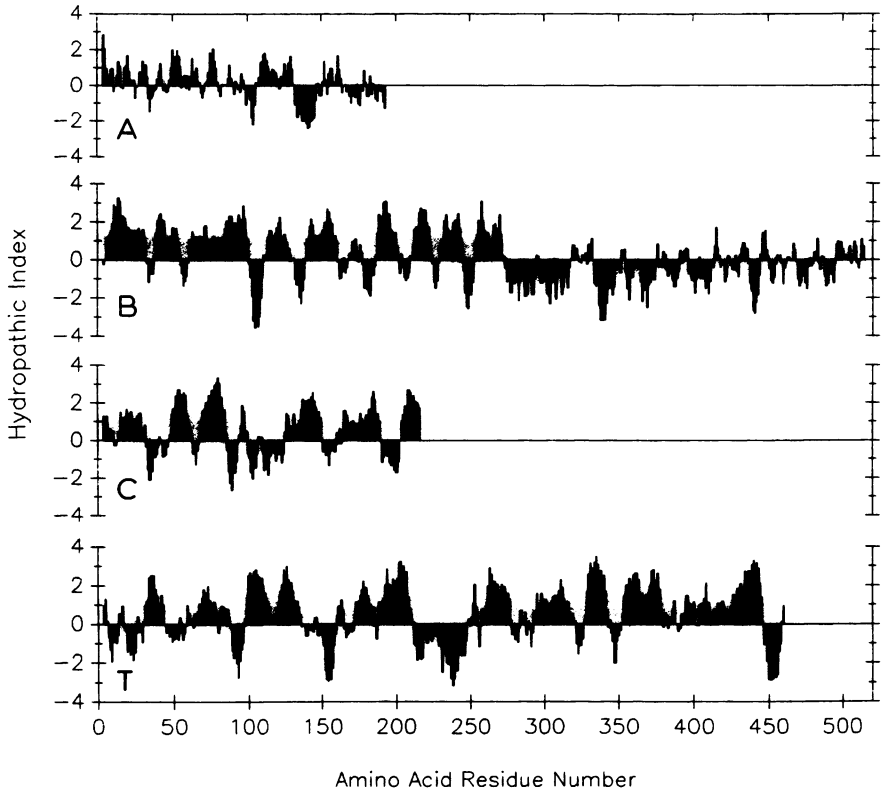


FIG. 3. Hydropathy profiles of the four Uhp polypeptides. The hydropathy parameters of Kyte and Doolittle (15) were averaged over a moving window of seven amino acids. The four polypeptides are (A) UhpA, (B) UhpB, (C) UhpC, and (T) UhpT. Values above the zero axis represent hydrophobic segments.

sequence TCAGGCCTGA is centered at the −35 position. Lying upstream from this region are numerous sequences of dyad symmetry and direct repeats, which may represent sites for binding of regulatory proteins. Further upstream, between nucleotides 3573 and 3593, at the area which lies at −97 to −117 relative to the transcript start site, is a typical catabolite activator protein-binding site. This sequence matches the consensus catabolite activator protein-binding site at 11 of 14 positions. Definition of the role of these sequences in promoter function is in progress. The absence of a canonical promoter sequence was expected for a promoter whose expression is dependent on a positive activator protein (20).

Codon usage. Grosjean and Fiers (11) noted a substantial difference in the distribution of codons between strongly and weakly expressed genes in *E. coli*. The preferences were ascribed to the optimization of the energy of the codon-anticodon interactions and to the amounts of the various isoaccepting tRNA species. So-called "modulator" codons are used rarely in highly expressed genes, whereas most of the codons are present in weakly expressed genes.

There are differences in the codon usage patterns of the four *uhp* genes which appear to be consistent with the expected differences in their levels of expression. The *uhpA*, *uhpB*, and *uhpC* genes contain fewer of the preferred codons, defined by Bennetzen and Hall (4), than does *uhpT*, i.e., 53, 48, and 46% versus 63%, respectively. Similarly, the three regulatory genes contain more modulator codons (6.1, 11.3, and 12.4%, respectively) than does *uhpT* (1.9%). Thus, the codon usage of *uhpT* is consistent with that of a relatively strongly expressed gene, whereas those of *uhpB* and *uhpC* are characteristic of weakly expressed genes and that of *uhpA* seems intermediate.

Another parameter of codon bias, termed P2′, was defined by Sharp and Li (24) and represents the preference for codons of intermediate base-pairing energy, i.e., those that contain both A-U and G-C pairings as opposed to those having only A-U or only G-C pairs. This P2′ parameter was proposed to be higher for more highly expressed genes. However, the P2′ values for the *uhp* genes were roughly the same, in contrast to the differences in codon bias seen with the parameters described above.

Characteristics of the predicted Uhp polypeptides. The properties of the Uhp polypeptides predicted from the nucleotide sequence compare favorably with the size and locations of the actual Uhp proteins observed in maxicells. UhpA is a soluble protein, whereas UhpB and UhpT are membrane associated. The predicted UhpA polypeptide has a content of charged amino acids typical of soluble proteins (Asp+Glu+Arg+Lys = 21 mol%), whereas the other three Uhp proteins are less polar (18.3, 12.8, and 14.5 mol% for UhpB, UhpC, and UhpT, respectively). UhpA has a net negative charge, and the other three proteins bear net positive charges.

Kyte and Doolittle (15) have developed a method for calculating the hydropathic character of a protein and for displaying the distribution of polar and nonpolar residues along its primary sequence. The average hydropathy values for UhpA and UhpB are 0.18 and 0.19, respectively, which are in the range of soluble proteins. UhpC and UhpT had more nonpolar character, with net hydropathy values of 0.59 and 0.56, respectively.

TABLE 1. Comparison of a portion of UhpA with DNA-binding regions of other regulatory proteins

Protein	Starting residue	Sequence[a]	Reference
UhpA	153	MAVKEIAAELGLSPKTVHVHRA	
cI	31	LSQESVADKMGMGQSGVGALFN	19
Cro	17	FGQTKTAKDLGVYQSAINLAIH	19
Cro 434	19	MTQTELATKAGVKQQSIQLIEN	10
TrpR	66	MSQRELKNELGAGIATITRGSN	
DeoR	22	LHLKDAAALLGVSEMTIRRDLN	30
CAP[b]	167	ILRQEIGQIVGCSRETVGRILK	27
AsnC	23	TAYAELAKQFGVSPGTIHVRVE	14
Fnr	195	MTRGDIGNYLGLTVETLSRLLG	27
OmpR	88	EVDRIVGLEIGADDYIPKPFNP	
PhoB	199	IRRLRKALEPGGHDRMVQTVRG	17

[a] Amino acid residues are in standard one-letter code. Residues in UhpA with a double underline are those in which the same residue is present in at least three of the other regulatory proteins; those with a single underline are replaced by homologous amino acids in at least seven of the other proteins.
[b] CAP, Catabolite activator protein.

Regions of high hydropathic character that are at least 20 amino acids in length might traverse the cytoplasmic membrane (8). The hydropathy profiles of the four Uhp proteins (Fig. 3) lead to definite predictions about the interaction of the proteins with the membrane. The UhpA protein has a hydropathy profile typical of a soluble protein, lacking long nonpolar stretches that might span the membrane. In contrast, UhpB, which is associated with the membrane fraction despite its overall polar character, consists of two distinct domains. The amino half of the protein is strongly nonpolar and has as many as nine potential membrane-spanning stretches. The carboxyl half of the protein is very polar and displays no regions of high hydrophobic character. Thus, it is likely that UhpB is embedded in the membrane by multiple traverses within its amino half, while the carboxyl half resides exclusively in either the periplasmic space or the cytoplasm.

The UhpC protein possesses four to six potential membrane-spanning segments. Based on this hydropathy profile and its overall nonpolar character, it is probable that UhpC is membrane associated, although this has not been demonstrated directly. The UhpT transport protein also contains numerous potential membrane-spanning regions distributed along its entire length, although it is more polar than other cytoplasmic membrane transport proteins such as the lactose permease (6) or the integral membrane components of periplasmic binding protein-dependent transport systems (2).

Homology of the Uhp proteins. Since the *uhpA* product appears to activate *uhpT* transcription (26, 32), it is likely that it binds to specific DNA sequences and hence might display sequence homologies with other DNA-binding proteins. DNA-binding proteins of known crystal structure, such as catabolite activator protein, Cro, cI, and TrpR, interact with their target sites through specific hydrogen bonds made by amino acid side chains present in a characteristic helix-turn-helix motif (19). Many other regulatory proteins possess sequence homologies to this bihelical region (10). Sequences homologous to this conserved 22-amino-acid region are found near the C terminus of UhpA (amino acid residues 153 to 174) and are compared with those of several other regulatory proteins in Table 1. Note that there is no obvious difference within this 22-amino-acid region between proteins that function as repressors and those that activate gene transcription. Of the regulatory proteins examined, UhpA shares the most amino acids in this region with AsnC, an activator of the *asnA* (asparaginase) gene (14).

The UhpT protein shares about 30% amino acid identity along its entire length with the GlpT glycerol 3-phosphate transport protein (W. Boos, personal communication). Both of these transporters appear to act by a phosphate-antiport mechanism (1). No substantial homology was seen between UhpT and any of the proteins present in the National Biomedical Research Foundation Protein Sequence Database.

A very striking homology exists between the regulatory protein UhpC and the transporter UhpT. In UhpC, this region of homology begins at amino acid residue 39 and continues to the end of the protein. This stretch matches UhpT between residues 160 and 345 and is characterized by 33% amino acid identity over the 185-amino-acid overlap. If one includes conservative amino acid substitutions, these sections of the two proteins are 66% homologous. Not only are the amino acid sequences conserved between these two proteins, but so too are their possible transmembrane orien-

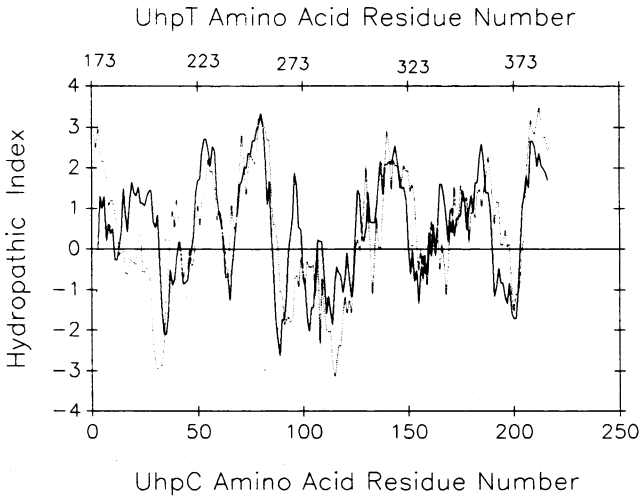


FIG. 4. Comparison of the hydropathy profiles of UhpC and the homologous portion of UhpT. The Kyte-Doolittle hydropathy profile of UhpC (solid line; coordinates on bottom ordinate) is compared with that of the region of UhpT with conserved amino acid sequence (dotted line; coordinates on top ordinate).

tations. The Kyte-Doolittle hydropathy distributions for UhpC and the corresponding portion of UhpT are portrayed in Fig. 4. The close correspondence within the homologous regions suggests that the two proteins have very similar conformations and orientations, although local differences exist.

The nucleotide sequence encoding the 185 conserved amino acids (aligned by five insertions of an amino acid in one or the other protein) exhibits 45.7% identity. The conservation of nucleotide sequence tends to be clustered and separated by stretches of unrelated sequence. Of the 60 identical amino acids present in analogous sites in the two proteins, more than half use the same codon. This suggests that the conserved portions of both proteins evolved from a common sequence.

The most likely mechanism for such identity is that the UhpC regulatory protein evolved by partial gene duplication from the UhpT transporter, whose synthesis it regulates. These two proteins could share similar structural requirements if one assumes that UhpC is the regulatory protein that binds the inducer and acts at the first step of the signaling process (32). Thus, both UhpC and UhpT proteins would have to span the membrane and possess a substrate-binding site accessible to the exterior. One factor that would prevent UhpT itself from serving this regulatory role is that its substrate specificity for transport function is much less stringent than the specificity for the inducer (reviewed in reference 32), arguing that the same binding site cannot be employed for both transport and regulation. To our knowledge, this is the first example where a regulatory protein might have evolved from the gene it regulates.

Implications for mechanism of regulation. One of the interesting features of the *uhp* system is its regulation by external inducer independent of UhpT transport function. The simplest model for exogenous induction involves the interaction of a transmembrane regulatory protein(s) with the inducer on the periplasmic face of the cytoplasmic membrane, which causes a transmembrane signaling event that activates the *uhpA* product to allow transcription of *uhpT*.

Properties of the predicted *uhp* polypeptides provide some support for this model. The UhpA activator protein contains a region with substantial homology to the areas of DNA-binding regulatory proteins that are responsible for the sequence-specific interactions between the protein and its DNA target (19). It remains to be determined where the UhpA protein binds in the *uhpT* promoter region and whether its activity is regulated by some covalent modification or by its release from the membrane.

Both UhpB and UhpC seem to be associated with the cytoplasmic membrane, although this has been directly shown only for UhpB. Both possess multiple stretches that have high hydropathic character and are at least 20 amino acids long, sufficient to span the membrane bilayer as an α helix. The amino-terminal half of UhpB could be almost totally embedded in the membrane, with the carboxy-terminal half folded on one side of the membrane. Predictions of the distribution of UhpB across the membrane suggest that few residues are exposed on the side of the membrane opposite the carboxyl half. These predictions suggest that UhpB has little transmembrane character, but rather that half is buried in the membrane and the other half is completely on one side of the membrane.

Hydropathy plots of UhpC are less amenable to prediction of transmembrane distribution, although several potential membrane-spanning regions are apparent. Substantial portions of this protein are expected to be exposed on either side of the membrane.

Genetic studies led to the proposal that UhpC might serve as receptor for the inducer. Mutations in *uhpA* or *uhpB* result in a Uhp^- phenotype that is rarely, if ever, reversed by second-site mutations. In contrast, the Uhp^- phenotype incurred by mutations in *uhpC* are suppressed by frequent events within another *uhp* gene or genes; the site of these mutations is unknown (13, 32). In most of these revertants, UhpT expression is no longer induced by the presence of glucose 6-phosphate. The strong homology of UhpC with UhpT is consistent with the presence on UhpC of a sugar-phosphate-binding site. Thus, our current model for regulation can account for the necessity for all three regulatory proteins by assuming that UhpB modifies UhpA in some way to convert it to an active form. The constitutive expression seen with the elevated copy number of *uhpA* is independent of UhpB and may reflect the increased equilibrium concentration of the activated conformer of UhpA. Direct evidence for several features of this model are being sought, and alternatives cannot be excluded.

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LITERATURE CITED

1. **Ambudkar, S. V., T. J. Larson, and P. C. Maloney.** 1986. Reconstitution of sugar phosphate transport systems of *Escherichia coli*. J. Biol. Chem. **261**:9083–9086.
2. **Ames, G. F.-L.** 1986. Bacterial periplasmic transport systems: structure, mechanism, and evolution. Annu. Rev. Biochem. **55**:397–425.
3. **Barr, P. J., R. M. Thayer, P. Laybourn, R. C. Najarian, F. Seela, and D. R. Tolan.** 1986. 7-Deaza-2′-deoxyguanosine-5′-triphosphate: enhanced resolution in M13 dideoxy sequencing. Biotechniques **4**:428–432.
4. **Bennetzen, J. L., and B. D. Hall.** 1982. Codon selection in yeast. J. Biol. Chem. **257**:3026–3031.
5. **Biggin, M. D., T. J. Gibson, and G. F. Hong.** 1983. Buffer-gradient gels and ^{35}S label as an aid to rapid DNA sequence determination. Proc. Natl. Acad. Sci. USA **80**:3963–3965.
6. **Buchel, D. E., B. Gronenborn, and B. Muller-Hill.** 1980. Sequence of the lactose permease gene. Nature (London) **283**: 541–545.
7. **Dale, R. M. K., B. A. McClure, and J. P. Houchins.** 1985. A rapid single-stranded cloning strategy for producing a sequential series of overlapping clones for use in DNA sequencing: application to sequencing the corn mitochondrial 18S rDNA. Plasmid **13**:31–40.
8. **Engelman, D. M., T. A. Steitz, and A. Goldman.** 1986. Identifying nonpolar transbilayer helices in amino acid sequences of membrane proteins. Annu. Rev. Biophys. Biophys. Chem. **15**: 321–353.
9. **Friden, P., J. Donegan, J. Mullen, P. Tsui, and M. Freundlich.** 1985. The *ilvB* locus of *Escherichia coli* K-12 is an operon encoding both subunits of acetohydroxyacid synthase I. Nucleic Acids Res.**13**:3979–3993.
10. **Gicquel-Sanzey, B., and P. Cossart.** 1982. Homologies between different prokaryotic DNA-binding regulatory proteins and between their sites of action. EMBO J. **1**:591–595.
11. **Grosjean, H., and W. Fiers.** 1982. Preferential codon usage in prokaryotic genes: the optimal codon-anticodon interaction energy and the selective codon usage in efficiently expressed

genes. Gene **18**:199–209.
12. **Kadner, R. J.** 1973. Genetic control of the transport of hexose phosphates in *Escherichia coli*: mapping of the *uhp* locus. J. Bacteriol. **116**:764–770.
13. **Kadner, R. J., and D. M. Shattuck-Eidens.** 1983. Genetic control of the hexose phosphate transport system of *Escherichia coli*: mapping of deletion and insertion mutations in the *uhp* region. J. Bacteriol. **155**:1052–1061.
14. **Kolling, R., and H. Lother.** 1985. AsnC: an autogenously regulated activator of asparaginase synthetase A transcription in *Escherichia coli*. J. Bacteriol. **164**:310–315.
15. **Kyte, J., and R. F. Doolittle.** 1982. A simple method for displaying the hydropathic character of a protein. J. Mol. Biol. **157**:105–132.
16. **Lipman, D. J., and W. R. Pearson.** 1985. Rapid and sensitive protein similarity searches. Science **227**:1435–1441.
17. **Makino, K., H. Shinagawa, M. Amemura, and A. Nakata.** 1986. Nucleotide sequence of the *phoB* gene, the positive regulatory gene for the phosphate regulon of *Escherichia coli* K-12. J. Mol. Biol. **190**:37–44.
18. **Messing, J.** 1983. New M13 vectors for cloning. Methods Enzymol. **101**:20–78.
19. **Pabo, C. O., and R. T. Sauer.** 1984. Protein-DNA recognition. Annu. Rev. Biochem. **53**:293–321.
20. **Raibaud, O., and M. Schwartz.** 1984. Positive control of transcription initiation in bacteria. Annu. Rev. Genet. **18**:173–206.
21. **Rosenberg, M., and D. Court.** 1979. Regulatory sequences involved in the promotion and termination of RNA transcription. Annu. Rev. Genet. **13**:319–353.
22. **Salser, W.** 1977. Globin mRNA sequences: analysis of base pairing and evolutionary implication. Cold Spring Harbor. Symp. Quant. Biol. **42**:985–1002.
23. **Sanger, F., S. Nicklen, and A. R. Coulson.** 1977. DNA sequencing with chain-terminating inhibitors. Proc. Natl. Acad. Sci. USA **74**:5463–5467.
24. **Sharp, P. M., and W. H. Li.** 1986. Codon usage in regulatory genes in *Escherichia coli* does not reflect selection for 'rare' codons. Nucleic Acids Res. **14**:7737–7749.
25. **Shattuck-Eidens, D. M., and R. J. Kadner.** 1981. Exogenous induction of the *Escherichia coli* hexose phosphate transport system defined by *uhp-lac* operon fusions. J. Bacteriol. **148**: 203–209.
26. **Shattuck-Eidens, D. M., and R. J. Kadner.** 1983. Molecular cloning of the *uhp* region and evidence for a positive activator for expression of the hexose phosphate transport system of *Escherichia coli*. J. Bacteriol. **155**:1062–1070.
27. **Shaw, D. J., D. W. Rice, and J. R. Guest.** 1983. Homology between CAP and Fnr, a regulator of anaerobic respiration in *Escherichia coli*. J. Mol. Biol. **166**:241–247.
28. **Shine, J., and L. Dalgarno.** 1974. The 3′ terminal sequences of *Escherichia coli* 16S rRNA: complementarity to nonsense triplets and ribosome binding sites. Proc. Natl. Acad. Sci. USA **71**:1342–1346.
29. **Staden, R.** 1980. A new computer method for the storage and manipulation of DNA gel reading data. Nucleic Acids Res. **8**:3673–3694.
30. **Valentin-Hansen, P., P. Hojrup, and S. Short.** 1985. The primary structure of the DeoR repressor from *Escherichia coli* K-12. Nucleic Acids Res. **13**:5926–5936.
31. **Wek, R. C., C. A. Hauser, and G. W. Hatfield.** 1985. The nucleotide sequence of the *ilvBN* operon of *Escherichia coli*: sequence homologies of the acetohydroxy acid synthase isozymes. Nucleic Acids Res. **13**:3995–4010.
32. **Weston, L. A., and R. J. Kadner.** 1987. Identification of Uhp polypeptides and evidence for their role in exogenous induction of the sugar phosphate transport system of *Escherichia coli* K-12. J. Bacteriol. **169**:3546–3555.
33. **Winkler, H. H.** 1970. Compartmentation in the induction of the hexose-phosphate transport system in *Escherichia coli*. J. Bacteriol. **101**:470–475.