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The Family of Organo-Phosphate Transport Proteins Includes a Transmembrane Regulatory Protein

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This review article briefly summarizes aspects of our current understanding of the Uhp sugar phosphate transport system in enteric bacteria, particularly the mode of genetic regulation of its synthesis. This regulation occurs by a process that involves an example of the very widespread and ever-growing group of so-called two-component bacterial regulatory systems, a mechanism of response to environmental signals that employs phosphate transfer reactions between constituent proteins. Of emphasis here is the unusual involvement in transmembrane signaling of the UhpC protein which is related in sequence and structure to some transport proteins, including the very protein whose synthesis it helps regulate.

KEY WORDS: Bacterial transport systems; transmembrane signaling; *E. coli*; Uhp system; protein kinases; membrane transport.

THE UHP SYSTEM

The Uhp transport system of *Escherichia coli* is an inducible, active transport system that allows uptake and utilization of a number of organo-phosphate compounds, including the phosphate esters of hexoses, pentoses, heptoses, amino sugars, and sugar alcohols. These compounds could be utilized as carbon and energy source if they are first dephosphorylated by a periplasmic phosphatase, transported by the appropriate sugar transport system, and rephosphorylated by intracellular kinases. The Uhp and related transport systems, which mediate the uptake of these compounds in unaltered form, represent an obviously more efficient metabolic scheme by conserving the phosphate bond energy. This type of transport system is rather erratically distributed among eubacteria. Inducible glucose-6-phosphate (Glu6P) transport has been found in many members of the Enterobacteriaceae (including *Escherichia*, *Salmonella*, *Enterobacter*, and *Serratia*) and in the gram-positive *Staphylococcus aureus* and *Streptococcus lactis*, but is absent from genera such as *Proteus*, *Pseudomonas*, *Corynebacterium*, and *Bacillus* (Winkler, 1973b). This broad distribution suggests that the natural substrates for these transport systems, such as sugar phosphates, are likely to be readily available in nature, particularly in the large intestine, in part through their release from sloughed cells of the intestinal epithelium, which turn over about every 2 to 3 days. Whereas the *S. aureus* version of this transport system carries both Glu6P and glycerol-3-P (Gly3P), *E. coli* possesses separate transport systems: UhpT for Glu6P and related compounds and GlpT for Gly3P (Maloney *et al.*, 1990).

The Uhp system has been found to display two unusual features of interest: its transport mechanism and the transmembrane regulation of its synthesis.

UHP TRANSPORT MECHANISM

It was known from work in the 70's, in large part from Herb Winkler's lab, that the Uhp system mediated accumulation of Glu6P in unaltered form, and that the accumulation was energy-dependent and

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blocked by exposure to protonophores (Winkler, 1973a; Essenberg and Kornberg, 1975). The impression that UhpT acts in the manner of a proton symport, analogous to the lactose permease, LacY, was dissipated by the incisive studies by Peter Maloney and his colleagues, which have been reviewed in Maloney *et al.* (1990) and which showed that UhpT and related transport systems employ a completely different mechanism. Their initial insight into the mechanism of energy coupling for sugar phosphate uptake came from study of cells of *Streptococcus lactis*, in which endogenous energy sources for transport are more readily depleted than in enteric bacteria. They demonstrated the presence of energy-independent Pi : Pi exchange (Pi = inorganic phosphate) and of sugar-P : Pi exchange. These same activities were found for the Glu6P/Gly3P uptake system in *S. aureus* and for GlpT and UhpT in *E. coli*, and were reproduced in proteoliposomes reconstituted with membrane proteins from the appropriately induced wild-type cells, but not from uninduced cells or the specific transport-deficient mutant. It appears that UhpT mediates the obligate electroneutral exchange of the monoanionic species of Pi with itself or with Glu6P and other organo-P substrates (Sonna *et al.*, 1988). Thus, Glu6P accumulation occurs by a secondary (perhaps tertiary) active transport process and is directly coupled to the downhill movement of Pi through the obligatory exchange properties of UhpT. The observed energy dependence of Glu6P uptake should reflect the energy requirements for the generation and maintenance of the transmembrane Pi gradient, which is either linked to the proton gradient via the Pit system, or coupled to ATP hydrolysis via the high-affinity but repressible Pst system.

The same obligatory anion exchange mechanism has been shown by Maloney's group to operate for GlpT and the phosphoglycerate transporter of *Salmonella typhimurium*, PgtP (Varadhachary and Maloney, 1991). It will be interesting to decipher the structural basis for the apparently profound difference in transport mechanism between the cation symport-coupled systems, such as LacY and the melibiose permease, MelB, and these obligate exchange systems. Note that other obligatory anion exchange systems have been described in bacteria and organelles, including the oxalate/formate exchanger from *Oxalobacter formigenes* (Ruan *et al.*, 1992), the adenine nucleotide translocator of mitochondria and *Rickettsia prowazekii* (Plano and Winkler, 1989), and the mitochondrial chloride/bicarbonate exchanger.

GENE ORGANIZATION OF *uhp* LOCUS AND STRUCTURE OF UhpT

Cloning of the genes for the transporters GlpT from *E. coli* (Eiglmeier *et al.*, 1987), PgtP from *S. typhimurium* (Goldrick *et al.*, 1988), and UhpT from both *E. coli* and *S. typhimurium* (Friedrich *et al.*, 1987; Island *et al.*, 1992) and determination of their nucleotide sequence allowed comparison of their primary sequences and prediction of their transmembrane topology. In the case of UhpT, the *uhp* locus at 82.1 min on the *E. coli* genetic map was cloned as a 6-kb DNA fragment by selecting for complementation of Uhp-negative mutants. There are four genes in the *uhp* locus, termed *uhpABCT*, all of which are very closely spaced with minimal intergenic distance. Mutational analysis showed that all four genes are essential for Uhp expression (Weston and Kadner, 1988; Island *et al.*, 1992). The gene organization of the *uhp* locus and some information about the sequence relatedness of the Uhp polypeptides are summarized in Fig. 1. The basis for identifying *uhpT* as the gene for the transporter protein included the findings that the Uhp$^-$ phenotype conferred by mutations in this gene could not be overcome by suppressor mutations, that Glu6P-transport activity in cells and reconstituted proteoliposomes was related to the *uhpT* gene dosage and expression (Ambudkar *et al.*, 1990), that only the *uhpT* transcript showed inducibility and varied in relation to the level of transport under

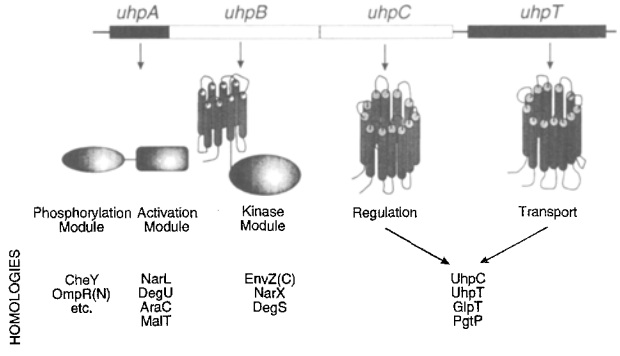

Fig. 1. Gene organization of the *uhp* locus. The 6-kb *uhp* locus from 82.1 min on the *E. coli* genetic map lies just downstream from the *ilvBN* operon, and is organized in two transcription units. The *uhpABC* genes are cotranscribed in the direction from left to right, and the *uhpT* gene is transcribed in the same direction from its own promoter between *uHpC* and *uhpT*. Below each gene is depicted a conceptual representation of each gene product, where the long cylinders represent transmembrane segments and the ellipses represent domains or modules in the cytoplasm. Below each protein or module are listed some of the other proteins with shared sequence homology.

various growth conditions, and that the *uhpT* coding region alone, when placed under control of a heterologous promoter, conferred Uhp transport activity in cells and reconstituted proteoliposomes (Ambudkar *et al.*, 1990; P. C. Maloney, personal communication).

As deduced from their nucleotide sequences, UhpT and GlpT are highly hydrophobic polypeptides of 463 and 452 amino acids, respectively. Based on hydropathy profiles and analysis of gene fusions to the topological reporter PhoA, both transporters are predicted to contain 12 transmembrane segments, oriented with the amino and carboxyl termini of the proteins facing the cytoplasm (Gött and Boos, 1988; Lloyd and Kadner, 1990). In neither case was the PhoA analysis exhaustive in testing the orientation of each potential extramembranous loop; one fusion to the putative last transmembrane segment of UhpT gave an anomalously low fusion activity that is not explained by the presence of positive charge near the fusion junction. An alignment of the sequences of UhpT from both enteric genera and GlpT is given in Fig. 2, from which it can be seen that both polypeptides are clearly and closely related along their entire lengths, with about 30% of the residues being identical in this alignment.

In the case of PgtP, the deduced sequence predicts a polypeptide of 406 amino acids, which is somewhat shorter than the others and ends within the 11th predicted transmembrane segment. Translation of the published nucleotide sequence (Goldrick *et al.*, 1988) shows the continuation after a frame shift of an open reading frame extending to the end of the sequence and showing reasonable sequence relatedness to the corresponding regions of UhpT and GlpT (indicated in Fig. 2 in lower case letters). It cannot be decided here whether this circumstance resulted from sequencing difficulties in the run of four T residues preceding the potential frame shift, from the requirement for a translational frameshift mechanism, or from an authentic truncation of the PgtP polypeptide by mutational insertion of a stop codon during evolution or cloning with retention of the untranslated downstream sequence. Comparison of the extended PgtP sequence with that of UhpT and GlpT shows that each polypeptide is about equally related to each other, ranging from 29.6 to 33.2% identical residues

Fig. 2. Alignment of the deduced nucleotide sequences of the *uhpC* and *uhpT* genes from *E. coli* and *S. typhimurium* (Island *et al.*, 1992), of GlpT from *E. coli* (Eiglmeier *et al.*, 1987), and of PgtP from *S. typhimurium* (Goldrick *et al.*, 1988). For the PgtP sequence, the amino acids at the carboxyl end encoded by the lower case letters are encoded in a different translational reading frame. The published reading frame for the authentic protein terminates with a Cys residue that follows the last upper case letter (F). Residues that are highly conserved and present in three or four of the four proteins are indicated by shading.

in the alignment shown in Fig. 2. Amino acid sequence conservation (shown by the PLOTSIMILARITY program of the University of Wisconsin GCG sequence analysis package) was maximal for the transmembrane segments, and this conservation was not simply the presence of hydrophobic residues, but reflected the presence of most of the invariant amino acid positions in this protein family. Thus, the overall transmembrane topology of the members of this family of transporter proteins is similar to that of LacY and other cation-coupled symporters.

REGULATION OF UHP EXPRESSION

The Uhp system is induced by the addition of Glu6P to the growth medium, but not by the Glu6P which is always present intracellularly, usually at concentrations in the millimolar range. Inducer specificity is much more stringent than the substrate specificity for transport, and induction responds to concentrations of Glu6P substantially lower than the K_m for transport (Winkler, 1970). This exogenous induction is expected because it is important for the regulatory system to ignore the constant presence of high levels of the inducing agent inside the cell, and thereby prevent formation of a transport system that could allow the fruitless loss of key phosphorylated metabolic intermediates. The use of *uhp-lac* fusions showed that regulation occurred at the transcriptional level, and that UhpT transport activity was not needed for efficient induction (Shattuck-Eidens and Kadner, 1981). Indeed, the presence of a functional transport system impaired the response to low levels of inducer by decreasing its external concentration. The degree of induction is striking, and the fold increase in β-galactosidase expression from a haploid *uhpT-lacZ* transcriptional fusion ranges from 500 to 1500, although the transcription in uninduced cells is too low to be measured reliably.

Analysis of transposon insertion mutations in each of the four *uhp* genes showed that the three genes upstream of *uhpT*, called *uhpABC*, were essential for induction of the *uhpT-lac* reporter (Weston and Kadner, 1988; Island *et al.*, 1992). Sequence comparisons of the Uhp regulatory proteins showed that UhpA and UhpB contain sequence motifs characteristic of members of the widespread group of two-component bacterial regulatory systems (Kofoid and Parkinson, 1988). The properties of two-component regulatory systems as cascades that use protein phosphorylation and phosphate transfer to signal transduction in response to environmental signals have been frequently reviewed (Parkinson and Kofoid, 1992). UhpA contains the amino-terminal phosphorylation module of about 120 amino acids that is characteristic of and highly conserved among response regulator proteins, while the carboxyl-terminal region is related to only a subfamily of transcription activator proteins. Sequence conservation in the carboxyl-terminal activation module is found among numerous transcription activators, some of which (NarL, DegU, FixJ, ComA, etc.) are parts of two-component regulatory systems, whereas others (LuxR, MalT, etc.) are apparently regulated by direct ligand binding and not by protein phosphorylation (Henikoff *et al.*, 1990). A potential helix-turn-helix-motif has been predicted to be present in the activation module of at least some members of this subfamily of proteins.

DOMAIN STRUCTURE OF UhpA

The transcription activator protein UhpA is absolutely required for Uhp expression, and its overproduction results in constitutive Uhp expression independent of the presence of functional forms of UhpB and UhpC (Weston and Kadner, 1988). The size of UhpA (196 amino acids) and its sequence homology patterns suggest the existence of two domains: at the amino end the phosphorylation module analogous to CheY (amino acids 1 to 120), and at the carboxyl end an activation/DNA-binding module (residues 135 to 196). To examine the action of the carboxyl-terminal region, we have prepared constructs encoding forms of UhpA truncated from that end by deletion of the nucleotide sequence at the 3′ end and replacement with translation termination signals. Removal of as few as eight amino acids resulted in a form of UhpA that not only was unable to activate *uhpT* transcription, but even interfered with the function of a resident wild-type version of UhpA. This same dominant-negative phenotype was seen in mutants removing additional residues up to residue 130. Further truncation to residue 118 or beyond eliminated this interference phenotype. These deletions extended into the phosphorylation module which is likely to be folded into the organized structure present in CheY (Volz and Matsumura, 1991). We are now investigating several possible mechanisms that could explain the interference

phenomenon incurred by overproduction of the phosphorylation module. One possibility is that the truncated species forms a mixed oligomer with the wild-type version of UhpA that is inactive for DNA binding or activation. Since the truncated version is present in excess to the wild-type form, the amount of functional multimer containing only the wild-type protein should be very low. Another possibility is that there is competition between the wild-type and truncated species for interaction with UhpB or another regulatory component. Distinction between these possibilities should be informative.

STRUCTURE OF UhpB

The carboxyl-terminal half of the UhpB polypeptide contains several sequence motifs that are highly conserved among the family of sensor-kinase proteins of two-component systems. These motifs include a putative ATP-binding region and segments surrounding an invariant asparagine residue and an invariant histidine residue that is the site of autophosphorylation, at least for NtrB (as reviewed in Parkinson and Kofoid, 1992). The amino-terminal portion of most sensor-kinase proteins is unique in sequence and often contains two transmembrane segments. This arrangement allows many sensor-kinase proteins to span the cytoplasmic membrane and present a large periplasmic domain that could be the site of interaction with the appropriate external signal, analogous to the structural model for the chemotaxis signal transducer proteins (Milburn *et al.*, 1991; Gardina *et al.*, 1992). A few sensor-kinase proteins which respond to intracellular signals, such as the state of fixed nitrogen, are cytoplasmic and lack membrane anchorage. The membrane-associated UhpB protein differs strikingly from this pattern in that its amino-terminal half (residues 1–273) is highly hydrophobic and could span the membrane up to 10 times. Our current model, based on hydropathy distribution, the presence of excess positive charge in cytoplasmic loops, the maintenance of charge neutrality within the membrane-spanning region, and limited PhoA fusion analysis favors the presence of 8 transmembrane segments, oriented so that the carboxyl-terminal kinase portion is located in the cytoplasm. By analogy with other two-component systems, we suspect that the presence of Glu6P in the medium triggers the phosphorylation pathway whereby UhpB phosphorylates itself on the invariant histidine residue and allows transfer of that phosphate to UhpA, which becomes activated to allow transcription at the *uhpT* promoter.

UhpC AND TRANSMEMBRANE SIGNALING

One of the most surprising conclusions from the sequence comparisons was the substantial sequence relatedness between the distal regulatory gene, UhpC, and the UhpT family of transporters. Following the correction of several sequencing errors and as shown in Fig. 2, the UhpC protein has 28–34% sequence identity to the three transporters, which is roughly the same degree of relatedness as the transporters share with each other. Although shorter by 20–30 amino acids, UhpC is predicted to have the same transmembrane topology and orientation as UhpT (Island *et al.*, 1992). Most significantly, this sequence relatedness extends over the entire length of these proteins, leaving no extended stretches of nonhomologous sequence that might be indicative of a unique signaling domain.

Is UhpC involved in the signaling process? Although direct demonstration of inducer binding to UhpC polypeptide has not yet been provided, several lines of genetic evidence support its role in regulation. As mentioned above, UhpC function is required for *uhpT-lac* transcription, but is not required for formation of an active transport system when *uhpT* expression is driven by a heterologous promoter, showing that UhpC is not a component of the transporter. Secondly, the Uhp$^-$ phenotype of strains carrying null mutations in *uhpC* can be suppressed by secondary mutations in the *uhp* locus, primarily causing amino acid substitutions in the carboxyl-terminal half of UhpB (B.-Y. Wei and R. J. Kadner, preliminary results). These suppressed mutants are invariably constitutive and insensitive to the presence of Glu6P, although the maximal level of expression depends on the identity of the suppressor mutation. This indirect result is consistent with the requirement for the presence of UhpC for responsiveness to Glu6P.

To gain more insight into the role of UhpC, we prepared a series of insertions of short, 4-codon oligonucleotide linkers in the *uhp* locus (Island *et al.*, 1992; Island and Kadner, 1993). These insertions were very easily constructed and localized within the *uhp* coding sequence by inserting a kanamycin-resistance cassette into DNA containing the *uhp* region that was opened by cleavage with frequently cutting

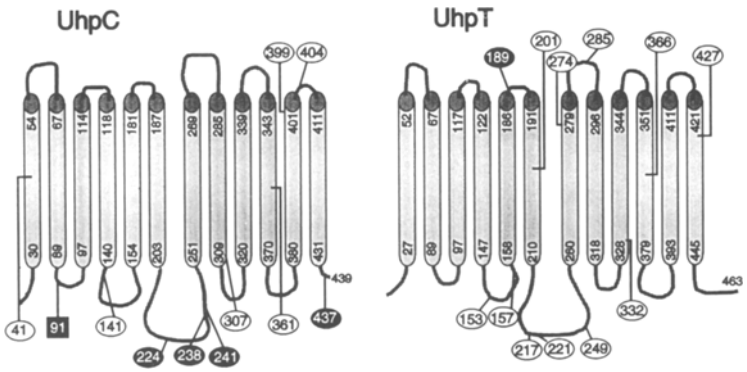

Fig. 3. Predicted transmembrane topology of the UhpC and UhpT polypeptides. The cylinders represent transmembrane segments, oriented so that the top faces the periplasm and the bottom faces inward to the cytoplasm. The numbers in the cylinders present the first and last amino acid residue in that segment. The numbers in ellipses or squares indicate the position of insertion of a 4-codon (12-bp) oligonucleotide linker. The insertions indicated by black numbers on a white background are Uhp$^-$ and unable to grow on sugar phosphates as carbon source and for the *uhpC* mutants to drive transcription of a *uphT-lac* reporter. The insertions indicated by white numbers on a black background are Uhp$^+$ and functional for transcription. Of the Uhp$^+$ insertions in *uhpC*, those represented by ellipses exhibit inducible regulation, and the insertion after amino acid 91 represented by a square exhibits high-level constitutive expression.

restriction enzymes. Removal of the cassette and religation leaves behind a short insert of defined sequence. Although the oligonucleotide sequence in each insert is the same, the nature of the encoded amino acids depends on the position in the translational reading frame. For the system used, however, all of the inserts encoded polar and charged residues, the possibilities being Asp-Leu-Gln-Val, Thr-Cys-Arg-Ser, or Ile-Arg-Arg-Arg, or the structure-disrupting sequence Gly-Pro-Ala-Gly-Pro. The functional consequences of 11 insertions in UhpC and 12 insertions in UhpT were determined from the growth response and transcriptional activity, in the case of UhpC.

As portrayed in Fig. 3, all but one of the linker insertions in UhpT resulted in loss of transport function. Protein stability and levels in the membrane have not been determined, and one expects that inserted charged residues in a transmembrane segment would interfere with the ability to fold properly in the membrane. However, seven of the UhpT mutants are predicted to be altered in an extramembranous loop, and only the insertion after residue 189 retained any activity. In contrast, about half of the 11 insertions in UhpC retained Uhp expression; all of these are predicted to occur in loops. Three insertions in the large, central cytoplasmic loop of UhpC were functional, with normal or near-normal levels of transcriptional control, whereas three insertions in the corresponding loop of UhpT were inactive. Two mutations inserted the same amino acids (Ile-Arg-Arg-Arg) at the homologous positions, after amino acid 241 of UhpC and 249 of UhpT. The insertion in UhpC was fully functional, but the one in UhpT was not. We conclude that UhpC function is more tolerant of amino acid changes than is UhpT, in particular, that the large, central cytoplasmic loop is important for transport by UhpT but not for regulation by UhpC. This conclusion is also consistent with the differences in amino acid sequence conservation when the deduced polypeptides from *E. coli* and *S. typhimurium* are compared: around 90% for UhpC, but almost 95% for UhpT (Island *et al.*, 1992). We suggest that UhpC does not carry out as coordinated or demanding a process as does UhpT; perhaps UhpC serves to bind inducer, but not transport it into the cell.

One of the Uhp$^+$ linker insertions in UhpC, after amino acid 91, had altered regulation and conferred high-level constitutive expression. The fact that a randomly generated mutation, made without selection for any particular phenotype, exhibited altered Uhp regulation, provides evidence for a role of UhpC in signaling.

UhpB AND UhpC IN SIGNALING

Genetic results suggestive of an interaction between UhpB and UhpC were obtained from the analysis of 14 linker insertions in *uhpB*. About half of these insertions were Uhp^+. Of six insertions that fell within the carboxyl-terminal half with the kinase and the phosphorylation motifs, four were inactive at stimulating *uhpT-lac* transcription; one had an elevated but noninducible basal level, and one was roughly wild-type in behavior. In contrast, six of the eight insertions in the amino-terminal half of UhpB between residues 1 and 273 were Uhp^+, although all showed altered regulation with uninduced levels increased anywhere from a slight degree to full constitutivity. These results indicate that mutational distortion throughout the transmembrane portion of UhpB can affect Uhp regulation in a manner that mimics receptor occupancy by the inducer, without interfering with the maximal level of inducibility. We suggest from these results that the membrane-embedded amino-terminal half of UhpB plays a direct role in Uhp signaling, and is not simply involved in holding UhpB at the membrane.

A surprising result was obtained when the UhpB insertions with constitutive behavior were combined with various *uhpC* mutations to test the epistatic relationships between the membrane-associated regulatory components. It was expected that the effect of most mutations affecting a component that acts early in a signaling pathway would not prevent the expression of mutations in a component acting later in the pathway. The constitutive expression conferred by most of the insertions in *uhpB* that affected the amino-terminal half occurred only in the presence of an active (inducible or constitutive) allele of *uhpC*. In the presence of an insertion that gave a negative phenotype in conjunction with $uhpB^+$ or a complete deletion of *uhpC*, there was no Uhp expression. The insertion at residue 151 of UhpB conferred constitutive Uhp expression regardless of the state of UhpC, showing that UhpC is not required for the integrity or stability of UhpB. Although the basis for this dependence of UhpB on UhpC function is not obvious, we conclude that these two proteins must interact in some way in the signaling process and that the contribution to regulation by UhpC is unlikely to be a product or discrete signal that is independent of UhpB.

It was observed that most Uhp^- mutants in *uhpB*, including a deletion of almost the entire coding sequence, resulted in an increased basal level of *uhpT* expression that was substantially higher than in the uninduced wild-type or in *uhpA* mutants, but which was not further induced by the presence of Glu6P (Island *et al.*, 1992; Kadner and Island, 1993). One explanation for this elevated basal level is that UhpB possesses both protein kinase and phosphatase activities, as has been found for some but not all of the sensor-kinase proteins of other two-component systems. We presume that in the uninduced state, the kinase activity of UhpB is inactive, but the phosphatase activity is available to reverse any adventitious phosphorylation of UhpA by other sensor-kinases. Addition of Glu6P should result in activation of the kinase function and inactivation of the phosphatase activity. Although both activities could be controlled independently, it would seem more parsimonious that they be commonly regulated but in opposite directions.

One mechanism for this control of UhpB activity would be through a conformational change coupled to the reception of the transmembrane signal reflecting the presence of external Glu6P. This conformational change conceivably could act either in a positive manner to activate the kinase function, or in a negative manner to relieve an inhibition of kinase activity. It is interesting to consider the possibility that regulation of UhpB is negative and that the kinase portion in the cytoplasmic carboxyl-terminal half of UhpB is inhibited by its interaction—direct or indirect—with segments in the nonpolar amino-terminal half of UhpB, or with UhpC, or with a complex of the two.

Evidence is not available to decide these questions, but some possibly mechanisms and analogies can be considered to explain why mutants affecting the membrane-embedded portion of UhpB can result in constitutive expression which in most cases is dependent on the presence of a functional version of UhpC. One model proposes that cytoplasmic loops formed by the transmembrane segments of UhpB or UhpC or both bind specifically to regions of the carboxyl-terminal portion of UhpB and thereby block its kinase activity or its ability to transfer its covalently bound phosphate to UhpA. The existence of inhibitory pseudo-substrate sites has been convincingly demonstrated in the cases of the eukaryotic protein kinases A and C, where part of either the same polypeptide chain or of a regulatory protein binds into the kinase's active site and thereby prevents its recognition of and reaction with authentic substrates (Cheng *et al.*, 1985; House and Kemp, 1987). For application of this model to the Uhp system, it is not necessary that the inhibitory interaction should involve a

pseudo-substrate site, for which no candidate sequences of similarity between segments in the amino-terminal half of UhpB and the phosphorylation sites of either UhpA or UhpB are obvious. It would only be necessary that the interaction with the extramembranous loops blocks some step of the phosphate transfer to UhpA, while allowing the phosphatase function to remain accessible and active.

A second model, analogous to that suggested for chemotactic transmembrane signaling, posits that a conformational change that alters the activity of the kinase and phosphatase functions in the carboxyl-half of UhpC is coupled to a conformational change that occurs in the amino-terminal half of UhpB when Glu6P is present. It is conceivable that the activating conformational change could be transmitted through the membrane-embedded portion of UhpB even if the Glu6P binds to UhpC, as long as UhpB and UhpC form a complex in the membrane, such that the relative movement of transmembrane segments in one protein elicits movement in contacting segments of the other protein.

A third possibility invokes changes in the state of oligomerization of UhpB as affecting its ability to be auto-phosphorylated. If phosphate is transferred from ATP bound to the kinase site on one UhpB monomer to the histidine residue on another UhpB monomer and if intramolecular phosphate transfer is not possible, then oligomerization of Uhp monomers, or at least their interaction, would be required for phosphorylation of UhpB. In such a situation, the ability of the UhpB monomers to associate might be affected, in either a positive or negative manner, by the presence or state of occupancy of UhpC. Changes in the oligomeric state of transmembrane-signaling protein kinases have been proposed for several eukaryotic receptors, such as the insulin receptor (summarized in Ullrich and Schlessinger, 1990). Experiments are in progress to test these various hypotheses and gain further information about the interaction of the Uhp regulatory proteins. One warning implicit in each of these models is that signaling is likely to be markedly sensitive to the stoichiometries of the various proteins, a possibility underscored by their gene organization, in which each of the three genes are organized in a way suggestive of their translational coupling.

CONCLUSIONS

The results presented in this brief summary, as well as many other observations on Uhp regulation, point out numerous interesting features. Not described here for space limitations is the question of the mechanism of transcription activation at the *uhpT* promoter. All of the nucleotide sequences required for normal transcriptional regulation are located with 130 bp upstream of the transcription start site (Merkel *et al.*, 1992). This region contains several familiar sequence elements, such as a typical -10 element for a σ^{70}-dependent RNA polymerase binding and a cAMP-receptor protein-binding site. This latter site allows strong activation of transcription, and the catabolite repressibility of Uhp expression is no doubt important to prevent various modes of toxicity that result from unrestrained transport of sugar phosphates (Kadner *et al.*, 1992). A sequence element of about 40 bp, centered at -64, has been implicated as the site for UhpA action. It is hoped that the apparent simplicity of the promoter region will make Uhp a useful system for study of transcription activation by response-regulators and for the interaction of two separate transcription-activating proteins.

Why is Uhp regulation dependent on the presence of a third protein, UhpC? Actually, most two-component systems use multiple components, as in the cases of Pgt, Pho, Ntr, etc. Perhaps the involvement of UhpC allows a greater degree of specificity in the recognition of the inducer than could be possible if only UhpB were the recognition element. In addition, this expanded system might allow a greater degree of signal amplification and sensitivity than possible in a two-component system, if one UhpC receptor molecule could activate multiple UhpB kinases in the regulatory cascade. At any rate, it is clear that alterations in the membrane-embedded portion of UhpB can activate Uhp expression, and that this response is affected by the presence of UhpC. The simplest explanations are that the amino-terminal part of UhpB interacts with UhpC in a signaling complex and negatively regulates UhpB function. Thus, although there appear to be three proteins involved in regulation, evidence so far suggests that they function as two components, with UhpC participating in regulation of UhpB activity; perhaps Uhp should be considered a 2.5-component system.

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