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A proposed link between nitrogen and carbon metabolism involving protein phosphorylation in bacteria

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Abstract

We demonstrate that certain phosphoryl transfer proteins of the bacterial phosphotransferase system (PTS), the fructose- and mannitol-specific IIA proteins or domains, are homologous to a class of proteins, one of which is known to affect transcription of some of the nitrogen-regulatory σ^{54}-dependent operons in *Klebsiella pneumoniae*. The phosphorylatable histidyl residue in the homologous PTS proteins and the consensus sequence in the vicinity of the active-site histidine are fully conserved in all members that comprise this family of proteins. A phylogenetic tree of the eight protein members of this family was constructed, and a "signature" sequence that can serve for the identification of new protein members of this family is proposed. These observations suggest that PTS-catalyzed protein phosphorylation may provide a regulatory link between carbon and nitrogen assimilation in bacteria.

Keywords: carbon metabolism; nitrogen metabolism; phosphotransferase system; protein phosphorylation; sigma factors

The bacterial phosphoenolpyruvate:sugar phosphotransferase system (PTS) detects, transports, and initiates the metabolism of many exogenous carbohydrates. In an earlier report we noted sequence similarity between the phosphorylatable active and regulatory regions of HPr, a phosphocarrier protein of the phosphotransferase system, and the molybdenum-iron protein of the nitrogenase complex from *Rhizobium trifolii* (Reizer, 1989). This observation led to the possibility that the PTS provided a link between carbon and nitrogen regulation, at least in some organisms. In the present communication we expand upon this suggestion. Our two laboratories have independently found that the fructose- and mannitol-specific IIA proteins or domains of the PTS (Saier & Reizer, 1992), i.e., IIA^{Fru} and IIA^{Mtl}, show striking sequence similarities, especially around a phosphorylatable histidyl residue, with open reading frames (ORFs) in several organisms that are encoded downstream from the nitrogen-regulating σ^{54} *rpoN* genes and which appear to negatively regulate the transcription of σ^{54}-regulated genes.

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Results

Figure 1 shows a multiple alignment of four PTS proteins and domains with the products of three homologous ORFs from three distinct bacteria. The four PTS proteins are the fructose-specific IIA domains (IIA^{Fru}) of *Salmonella typhimurium* and *Rhodobacter capsulatus* (Geerse et al., 1989; Wu et al., 1990) and the homologous mannitol-specific IIA proteins of *Escherichia coli* and *Staphylococcus carnosus* (Lee & Saier, 1983; Fischer et al., 1989). The three ORFs that are encoded downstream of the *rpoN* gene have been designated ORF162 of *Klebsiella pneumoniae* (Merrick & Coppard, 1989), ORF>88 of *Pseudomonas putida* (Inouye et al., 1989; Merrick & Coppard, 1989), and ORF>90 of *Bradyrhizobium japonicum* (Kullik et al., 1991). Although a recent study has indicated that the product of ORF162 regulates the activity of σ^{54} in *K. pneumoniae*, the latter two ORFs have been only partially sequenced, and their roles are not yet estab-

lished. Among the 11 fully conserved residues in all of these proteins (boxed in Fig. 1), histidine$_{73}$ in PTS members of this family (boxed and shaded in Fig. 1) is known to be phosphorylated by the general phosphocarrier protein, HPr (Reiche et al., 1988; van Weeghel et al., 1991). Furthermore, 6 of the 11 fully conserved residues cluster in the immediate vicinity of this phosphorylatable histidine. The conserved arginine–glutamate peptide sequence (positions 57–58) in this cluster is likely to play a catalytic role, whereas the other fully conserved residues (glycine, alanine, proline, and threonine) probably play a structural role. Downstream from the active-site histidine$_{73}$ is a histidyl residue (position 120), which is fully conserved in all proteins that have been sequenced in this region. Based on the known biochemical properties and the atomic structure of PTS proteins that comprise the IIA^{Glc} family (Presper et al., 1989; Liao et al., 1991; Fairbrother et al., 1992; Reizer et al., 1992), we propose that this second conserved histidyl residue interacts with the active-site phosphoryl group acceptor, i.e., histidyl$_{73}$, and is essential for transfer of the phosphoryl group from IIA^{Mtl} or IIA^{Fru} to the IIB domains of the mannitol- and fructose-specific PTS permeases, respectively. It is conceivable that by analogy to the IIA proteins or domains of the PTS, the regulation of σ^{54} activity involves phosphorylation of ORF162, ORF>88, and ORF>90 at the histidyl residue equivalent to histidine$_{73}$ in the aligned proteins and that histidine$_{120}$ plays an essential role in the transfer of phosphoryl groups from the active-site histidine to as yet unidentified proteins. This scenario is further supported by the previously published report indicating a constitutively low expression and cotranscription of ORF162 and *rpoN* (Merrick & Coppard, 1989). Thus, under physiological conditions, the regulatory role(s) of ORF162 in *K. pneumoniae*, and of its homologous counterpart proteins in other bacteria, may be mediated in part by a phosphorylation mechanism.

Examination of the fully conserved residues in the seven proteins aligned in Figure 1 and computer analyses of these sequences revealed a signature sequence that is present in all members of this protein family but is absent from all other proteins in the current PIR (version 29) and SWISSPROT (version 19) databanks. This sequence is:

REXXXXTX(LIVMF)GX(GST)(LIVM)A(LIVM)PH(GC)

(ambiguous residues at a specific position are given in parentheses, whereas any amino acid at a position in which

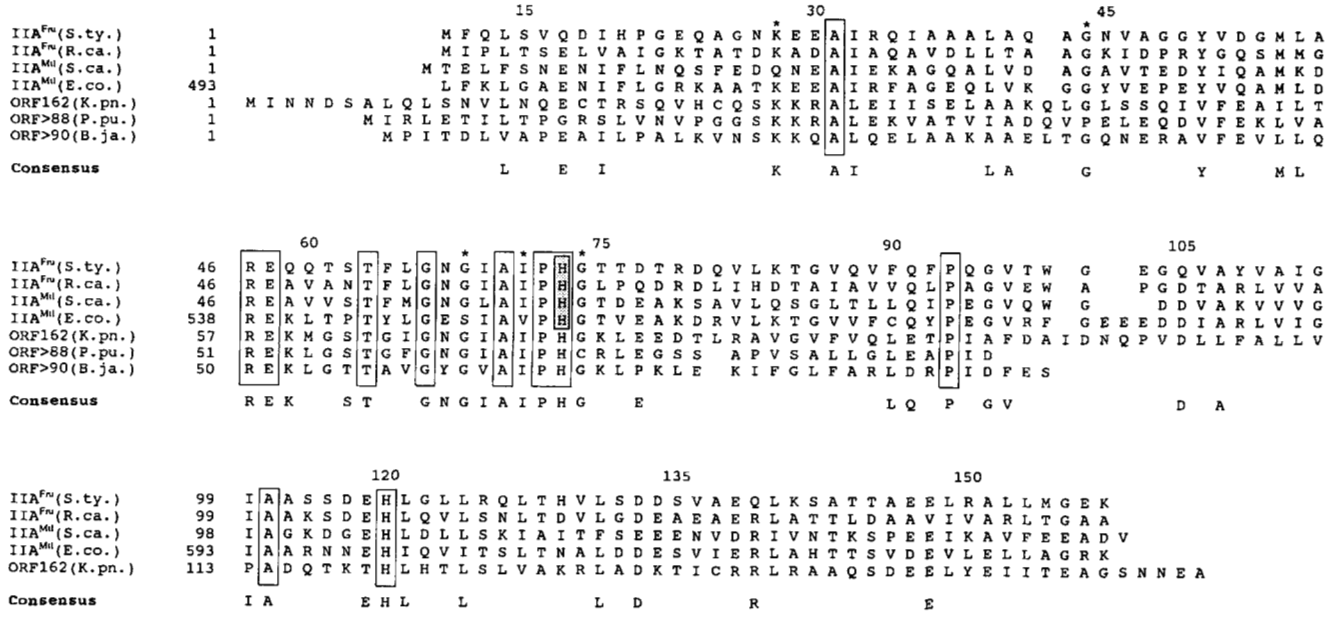

Fig. 1. Multiple alignment of the seven sequenced members of the family, which include the IIA^{Fru} and IIA^{Mtl} PTS proteins (or domains) and the putative regulatory proteins of σ^{54} action. Conserved residues in all fully or partially sequenced members of this family are boxed. The active-site histidine residue (site of phosphorylation) in the PTS proteins is doubly boxed and shaded. Asterisks denote residues common to all but one of the seven aligned proteins. Residues common to at least four of the seven sequenced proteins are shown in the consensus sequence (Consensus). Numbers to the left of the sequences shown refer to the first amino acid in the row. Numbers above the aligned sequences correspond to the positions of alignment and not to the residue numbers in any one of the aligned proteins. Abbreviations are as follows: B.ja., *Bradyrhizobium japonicum*; E.co., *Escherichia coli*; K.pn., *Klebsiella pneumoniae*; P.pu., *Pseudomonas putida*; R.ca., *Rhodobacter capsulatus*; S.ca., *Staphylococcus carnosus*; S.ty., *Salmonella typhimurium*.

Table 1. *Binary comparisons of the alignments of the mannitol- and the fructose-specific IIA proteins of the PTS with the putative regulatory proteins of σ^{54} activity*

	IIA^{Mtl} (S.ca.) (143)[a]	IIA^{Fru} (S.ty.) (143)	IIA^{Fru} (R.ca.) (143)	ORF>90 (B.ja.) (>90)	ORF>88 (P.pu.) (>88)	ORF162 (K.pn.) (162)
IIA^{Mtl} (E.co.) (145)	39 (138)[b] [31]	45 (145) [40]	38 (143) [32]	27 (70) [9]	36 (33) [5]	27 (105) [14]
IIA^{Mtl} (S.ca.) (143)		37 (36) [30]	35 (139) [33]	25 (64) [5]	29 (77) [7]	25 (114) [6]
IIA^{Fru} (S.ty.) (143)			42 (141) [34]	27 (70) [9]	39 (49) [8]	24 (138) [9]
IIA^{Fru} (R.ca.) (143)				27 (67) [4]	25 (60) [4]	26 (106) [9]
ORF>90 (B.ja.) (>90)					36 (87) [18]	36 (91) [21]
ORF>88 (P.pu.) (>88)						44 (87) [20]

[a] The values in parentheses below the protein designations indicate the number of amino acids in the protein.
[b] Values reported in the table correspond to the percent identity in the segments compared. The number of amino acids in the aligned segment is provided in parentheses. Values in brackets denote the comparison score in standard deviations higher than those obtained with 200 comparisons of randomized sequences of these protein segments. The FASTA and RDF2 programs (Pearson & Lipman, 1988) were used to assess the similarity scores and their significance. The abbreviations used are the same as in Figure 1.

the residue is not specified is denoted by X). This signature sequence should assist in the identification of new protein members of this family.

The statistical analyses of these protein sequences are summarized in Table 1. The percent identities and comparison scores (in standard deviations, SD) establish that all of these proteins are homologous, i.e., are derived from a common ancestral protein. Particularly worthy of note are the facts that (1) the binary comparison scores of the IIA proteins are in the range of 30–40 SD, (2) the binary comparison scores of the ORFs are between 18 and 21 SD, and (3) the comparison score for IIA^{Mtl} of *E. coli* with ORF162 of *K. pneumoniae* is 14 SD. These values are of sufficient magnitude to establish that all seven proteins comprise a new family (Doolittle, 1981). The partial sequences of two additional proteins that most likely share similar properties with the proteins described here have been recently reported. Thus, the sequence flanking the active-site histidyl residue in IIA^{Mtl} of *Staphylococcus aureus* was shown to be the same as that of IIA^{Mtl} of *S. carnosus* (Reiche et al., 1988), and the seven sequenced N-terminal residues of an ORF downstream of the gene encoding σ^{54} of *Salmonella typhimurium* was shown to be similar to the N-terminal residues of the *K. pneumoniae* ORF162 (Popham et al., 1991).

The phylogenetic tree for these proteins is shown in Figure 2. The four PTS proteins clearly cluster together, and the three putative nitrogen regulatory proteins (ORFs) form a distinct but less cohesive cluster. It should be noted that the apparent distances between the proteins in the latter cluster are likely to diminish when the complete sequences are available.

Discussion

It has been reported that species of *Rhizobium* lack a functional PTS for glucose (San Francisco & Jacobson, 1986; Glenn et al., 1984). In view of the sequence com-

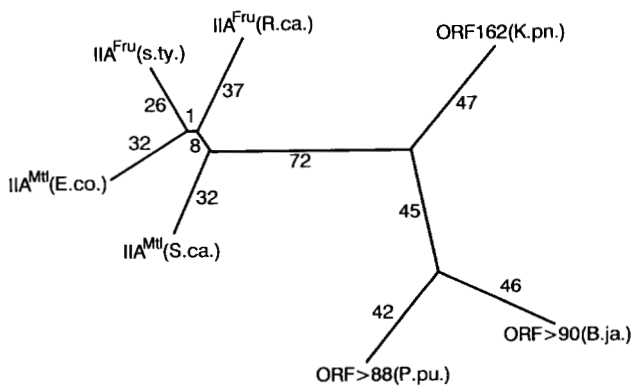

Fig. 2. Phylogenetic tree of the seven sequenced members of the family, which includes the IIA^{Fru} and IIA^{Mtl} proteins or domains of the PTS and the putative regulatory proteins that influence σ^{54} action. The relative branch lengths are shown adjacent to the branches. Abbreviations are as indicated in the legend to Figure 1. The progressive alignment method was used to construct the phylogenetic tree (Feng & Doolittle, 1990).

parisons reported here, it is possible that these organisms possess at least the general energy coupling proteins of the PTS, Enzyme I and HPr, and that these proteins play a role in the phosphorylation and consequent regulatory function(s) of the ORFs of *B. japonicum, K. pneumoniae*, and *P. putida*, which appear to regulate σ^{54}-dependent transcriptional initiation (Merrick & Coppard, 1989). The PTS could thus serve as the novel protein kinase postulated by Schneider et al. (1991). We note that the presence of Enzyme I and/or HPr in bacteria lacking a functional PTS is not without precedence since the presence of one or both of these general energy coupling proteins was recently demonstrated in *Lactobacillus brevis* (Reizer et al., 1988), *Acholeplasma laidlawii* (Reizer et al., unpubl.), and *Alcaligenes eutrophus* (Pries et al., 1991). Because σ^{54} is frequently, but not always, concerned with nitrogen regulation (Kustu et al., 1989; Thony & Hennecke, 1989) it is possible that PTS-catalyzed phosphorylation of these regulatory proteins plays a role in coordinating carbon and nitrogen assimilation. Our previous report, showing that HPr of the PTS exhibits sequence similarity to nitrogenase of *R. trifolii* (Reizer, 1989), substantiates this suggestion. Moreover, we have found that the *S. typhimurium* diphosphoryl transfer protein (DTP; Geerse et al., 1989; Wu et al., 1990; Saier & Reizer, 1992) exhibits sequence similarity (40% identity in a 50-residue segment with a comparison score of 8.2 SD) with the NodC protein of *Rhizobium loti* (Collins-Emerson et al., 1990). Further, the recent demonstration (Dèbarbouillè et al., 1991) that the LevR protein of *Bacillus subtilis* has domains homologous both to activators of σ^{54}-dependent transcription (i.e., NifA and NtrC) and to PTS-dependent transcriptional regulators (i.e., SacT, SacY, and BglG) provides further support for our suggestion that PTS-catalyzed protein phosphorylation provides a regulatory link between carbon and nitrogen assimilation. Molecular genetic and biochemical experiments are currently underway to examine the functional consequences of the sequence similarities noted in this communication.

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