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Regulatory Interactions Involving the Proteins of the Phosphotransferase System in Enteric Bacteria

Milton H. Saier, Jr.

Department of Biology, University of California at San Diego, La Jolla, California 92093-0116

Abstract Sugar uptake and cytoplasmic inducer generation as well as cyclic AMP synthesis are regulated by the phosphoenolpyruvate:sugar phosphotransferase system (PTS) in Gram-negative enteric bacteria. In these organisms, the free form of the glucose-specific Enzyme IIA (IIA^{glc}) of the PTS, which can be phosphorylated on a histidyl residue by PEP and the PTS energy coupling proteins, inhibits the activities of non-PTS carbohydrate permeases and catabolic enzymes. By contrast, the phosphorylated form of IIA^{glc} appears to activate adenylate cyclase, the cyclic AMP biosynthetic enzyme. What is known of the molecular details of these regulatory interactions will be summarized, and a novel regulatory mechanism involving the fructose repressor, FruR, which controls the transcription of genes encoding enzymes which catalyze reactions in central pathways of carbon metabolism, will be presented.

Key words: enteric bacteria, phosphotransferase system, protein phosphorylation, transport regulation, metabolic regulation, inducer exclusion, adenylate cyclase, transcriptional regulation, fructose represser

Carbohydrates are transported into bacteria by a multiplicity of mechanisms, one of which depends on the catalytic activities of the protein constituents of the phosphoenolpyruvate (PEP): sugar phosphotransferase system (PTS). In *Escherichia coli*, the PTS is also known to control the activities of certain non-PTS carbohydrate permeases (i.e., those for lactose, melibiose, and maltose), as well as the glycerol catabolic enzyme, glycerol kinase, and the cyclic AMP biosynthetic enzyme, adenylate cyclase [Saier, 1989a; Saier et al., 1990]. In *E. coli* a sugar-specific PTS protein, the glucose-specific Enzyme IIA (IIA^{glc}) [Saier and Reizer, 1992], is phosphorylated by a PEP-dependent mechanism on a histidyl residue, and only the free (dephospho) form of IIA^{glc} binds to the allosteric site on a cytoplasmic catabolic enzyme or on the cytoplasmic face of an integral membrane permease protein to inhibit its activity. By contrast, adenylate cyclase, the cyclic AMP biosynthetic enzyme, is apparently activated by phospho-histidyl IIA^{glc} [Saier, 1989a]. Recently, the three-dimensional structure of the allosteric regulatory protein of the PTS, IIA^{glc}, has been determined both by X-ray crystallography and by two- and three-dimensional NMR (see the article by Chen et al. this issue). Further, mutational analyses have provided information regarding the protein-protein interaction sites. In this brief review, the current status of research on this regulatory mechanism will be presented, and a novel mechanism of transcriptional regulation, possibly involving PTS-mediated phosphorylation events, will be presented.

ALLOSTERIC REGULATION OF CARBOHYDRATE PERMEASES, CATABOLIC ENZYMES, AND ADENYLATE CYCLASE

The involvement of the PTS as a protein phosphorylating system in the regulation of gene transcription and metabolism in bacteria has been discussed in earlier reviews [Saier, 1989a,b; Saier et al., 1990]. The literature up through 1988 on the regulatory mechanism by which the IIA^{glc} protein of enteric bacteria controls the activities of non-PTS permeases such as the lactose, melibiose, and maltose permeases, as well as the cytoplasmic enzymes, glycerol kinase and adenylate cyclase, has been reviewed in detail [Saier, 1989a]. The essential features of the regulatory mechanism involve the IIA^{glc} protein [Saier and Reizer, 1990, 1992] which is reversibly phosphorylated by PEP, Enzyme I, and HPr of the PTS and is believed to allosterically control the activities of all of the target proteins. Based primarily on genetic and physiological experiments, adenylate cyclase appears

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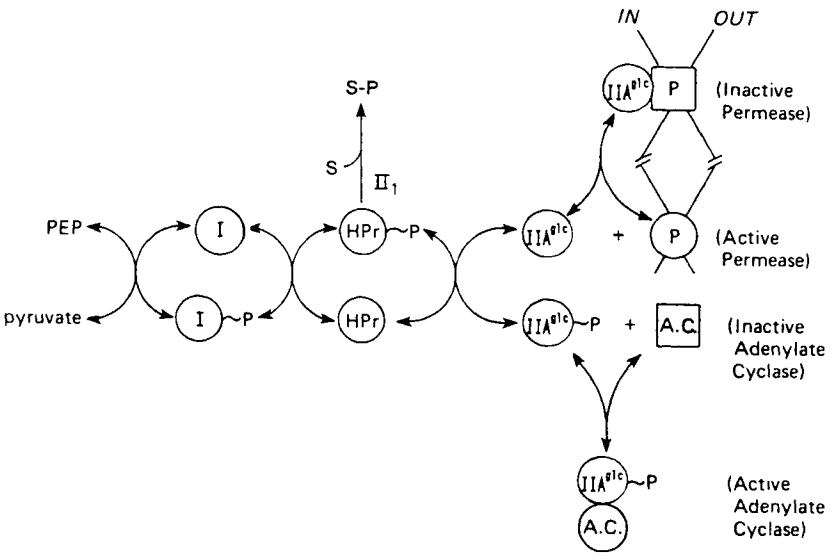


Fig. 1. Regulatory scheme involving the reversible phosphorylation of the IIA^{glc} protein of the PTS in enteric bacteria such as *E. coli* and *S. typhimurium*. I, Enzyme I; HPr, heat stable phosphocarrier protein of the PTS; IIA^{glc}, the Enzyme IIA^{glc} protein; P, permease (or glycerol kinase); AC, adenylate cyclase (modified from Saier, 1977, with permission).

MalK	275	Val	Gln	- - -	Val	**Gly**	**Ala**	Asn	Met	Ser	Leu	- - -	**Gly**	Ile	Arg	Pro
		.	.		:	:	:	:		:	.			.	.	
LacY	198	**Ala**	Asn	Ala	Val	Gly	Ala	Asn	His	Ser	Ala	Phe	**Ser**	Leu	Lys	Leu

Fig. 2. Sequence comparison between regions of the MalK and LacY proteins which include residues (indicated in bold print) which when mutated abolish the binding of IIA^{glc} to the maltose and lactose permeases of *E. coli*, respectively. The two sequences exhibit striking similarity as shown. The probability that this degree of sequence identity arose by chance is less than 10^{-4}. Since these two proteins are not homologous, convergent evolution for a common function (presumably binding of IIA^{glc}) probably was responsible for the sequence similarity observed (modified from Dean et al., 1990 with permission).

to be allosterically activated by phosphohistidyl IIA^{glc}. Detailed biochemical work has established that the permeases and glycerol kinase are allosterically inhibited by free IIA^{glc} as outlined in Figure 1. Binding of the IIA^{glc} protein to the target permease or enzyme is apparently cooperative with substrate binding [Osumi and Saier, 1982b; Nelson et al., 1984, 1986; Novotny et al., 1985].

Three recent advances in the definition of the detailed regulatory mechanism have been 1) to identify residues in target permeases which when mutated abolish binding of IIA^{glc} to the allosteric site, 2) to determine the three-dimensional structure of IIA^{glc}, and 3) to identify residues in IIA^{glc} which when mutated abolished the inhibition of permease function. The results of the first of these studies [Dean et al., 1990; Wilson et al., 1990; Kühnau et al., 1991] identified regions in the lactose permease (LacY) and the maltose permease (the MalK protein) which are believed to bind IIA^{glc} (see Fig. 2). These regions in the two permease proteins show a surprising degree of sequence similarity considering that LacY and MalK are non-homologous proteins. Thus, LacY is the sole integral membrane constituent of the lactose permease while MalK is a peripheral membrane protein which is only one of several constituents of the maltose permease. The probability that the degree of sequence similarity in the regions of these two permeases shown in Figure 2 [Dean et al., 1990] arose by chance is less than one in 10,000. This fact leads to the suggestion that the sequence similarity observed arose by convergent evolution as a result of a need for a common function (i.e., the binding of IIA^{glc}). More detailed analyses of mutations in LacY [Roepe et al., 1990] which alter IIA^{glc} binding are currently in progress.

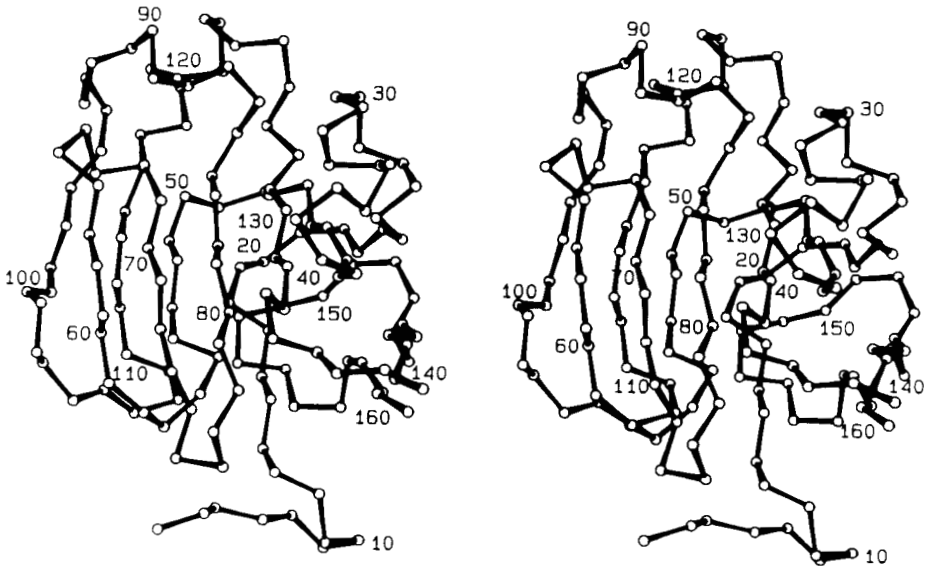
The three-dimensional structure of IIA^{glc} is shown in Figure 3 [Liao et al., 1991; Fairbrother et al., 1992] and discussed in greater detail in the subsequent article [Chen et al., 1992]. This heat stable protein, which can be autoclaved

(121°) without complete loss of activity [Sutrina et al., 1990; Reizer et al., 1992], possesses the essence of an anti-parallel β-barrel with Greek key and jelly roll topological features [Branden and Tooze, 1991; Liao et al., 1991; Fairbrother et al., 1992]. In Figure 3A, the ribbon structure of IIA^{glc} is presented with emphasis on secondary structural elements. β strands are presented as ribbons while helices are shown as cylinders. The latter are present in the loop regions. The active site histidyl residue which can accept a phosphoryl group from phospho HPr is located at the C-terminal end of a β strand. In Figure 3B, the space-filling model of IIA^{glc} is presented with the same orientation as in A. The two histidyl side chains at the active site, shown at the top third of the molecule, are perpendicular to each other (see figure legend). Knowledge of this three-dimensional structure should allow precise definition of the regions of the protein which interact with target systems.

Recently Zeng et al. [1992] have isolated and sequenced mutant crr genes in which IIA^{glc} is specifically altered so that transport regulation by this protein is abolished, even though the activity of phospho IIA^{glc} in activating glucose uptake and phosphorylation as well as cyclic AMP synthesis was not impaired. Each of three mutations which were characterized was shown to be due to a single amino acid substitution as follows: $Gly_{47} \rightarrow Ser$ (G47S); $Ala_{76} \rightarrow Thr$ (A76T), and $Ser_{78} \rightarrow Phe$ (S78F). The identification of these altered residues in the mutant proteins implicates them in the binding of IIA^{glc} to the target permeases. Examination of the three-dimensional structure of IIA^{glc} reveals that A76 and S78 are within the β strand that bears one (H75) of the two essential histidines (H75 and H90), which together in part comprise the active site of the protein [Liao et al., 1991; Worthylake et al., 1991; Fairbrother et al., 1992]. H75 is a catalytic residue while H90 is the phosphorylation site. A76 and S78 therefore probably comprise part of the active site. While G47 is fully conserved in all IIA^{glc}-like proteins or protein domains sequenced to date, and A76 is conserved in all but one of them, S78 is only found in the IIA^{glc} proteins from enteric bacteria [Liao et al., 1991]. This last residue may therefore be of importance only for the regulatory functions of IIA^{glc}. Since the A76T and S78F mutations both introduce more bulky side chains into the active site, they may alter its conformation or directly block the allosteric protein-protein interactions. The results suggest that the permeases bind IIA^{glc} in the region of the latter protein's active site. The fact that the G47S mutation also alters the regulatory interaction may be more difficult to interpret. The conserved glycine at position 47 undoubtedly plays an essential structural role, and consequently the G47S substitution may substantially alter the overall structure of IIA^{glc}. It is interesting to note that preliminary information suggests that the region of IIA^{glc} to which the lactose permease probably binds overlaps the HPr binding site (Y. Chen, J. Reizer, M.H. Saier, Jr. and P.E. Wright, unpublished results). This observation is surprising in view of the early report that IIA^{glc} can bind the lactose permease and HPr simultaneously [Osumi and Saier, 1982a].

PHYSIOLOGICAL CONSEQUENCES OF IIA^{glc}-MEDIATED REGULATION

What are the physiological consequences of IIA^{glc}-mediated regulation? As a result of extensive genetic and physiological analyses of the IIA^{glc}-mediated regulatory system, its functional consequences are well defined. Transcription of the lactose (*lac*), maltose (*mal*), melibiose (*mel*), and glycerol (*glp*) regulons is subject to dual control mediated by inducer, acting together with a carbohydrate-specific repressor protein, as well as cyclic AMP, acting together with the cyclic AMP receptor protein (CRP). It would therefore make sense for cytoplasmic inducer and cyclic AMP levels to be coordinately regulated. Moreover, since PTS sugars are generally preferred to non-PTS sugars because of energetic considerations, one would expect that the former sugars would be utilized preferentially to the latter sugars (i.e., PTS sugars would be at the top of the heirarchy while non-PTS sugars would be nearer the bottom). The IIA^{glc}-mediated regulatory mechanism insures that this relationship is maintained by allowing PTS sugars to cause inhibition of cytoplasmic inducer generation as well as cyclic AMP synthesis. This is achieved because a PTS sugar in the extracellular medium, when transported into the cell, utilizes the phosphoryl group of phospho histidyl HPr for its own phosphorylation, and since IIA^{glc}/phospho IIA^{glc} is in equilibrium with HPr/phospho HPr, this causes IIA^{glc}-phosphate to become dephosphorylated. Loss of cytoplasmic phospho IIA^{glc} deactivates adenylate cyclase, and generation of the dephospho form of IIA^{glc} causes inhibition of the non-PTS carbohydrate permeases and glycerol kinase. Consequently, cytoplasmic levels of cyclic AMP and inducer decrease,



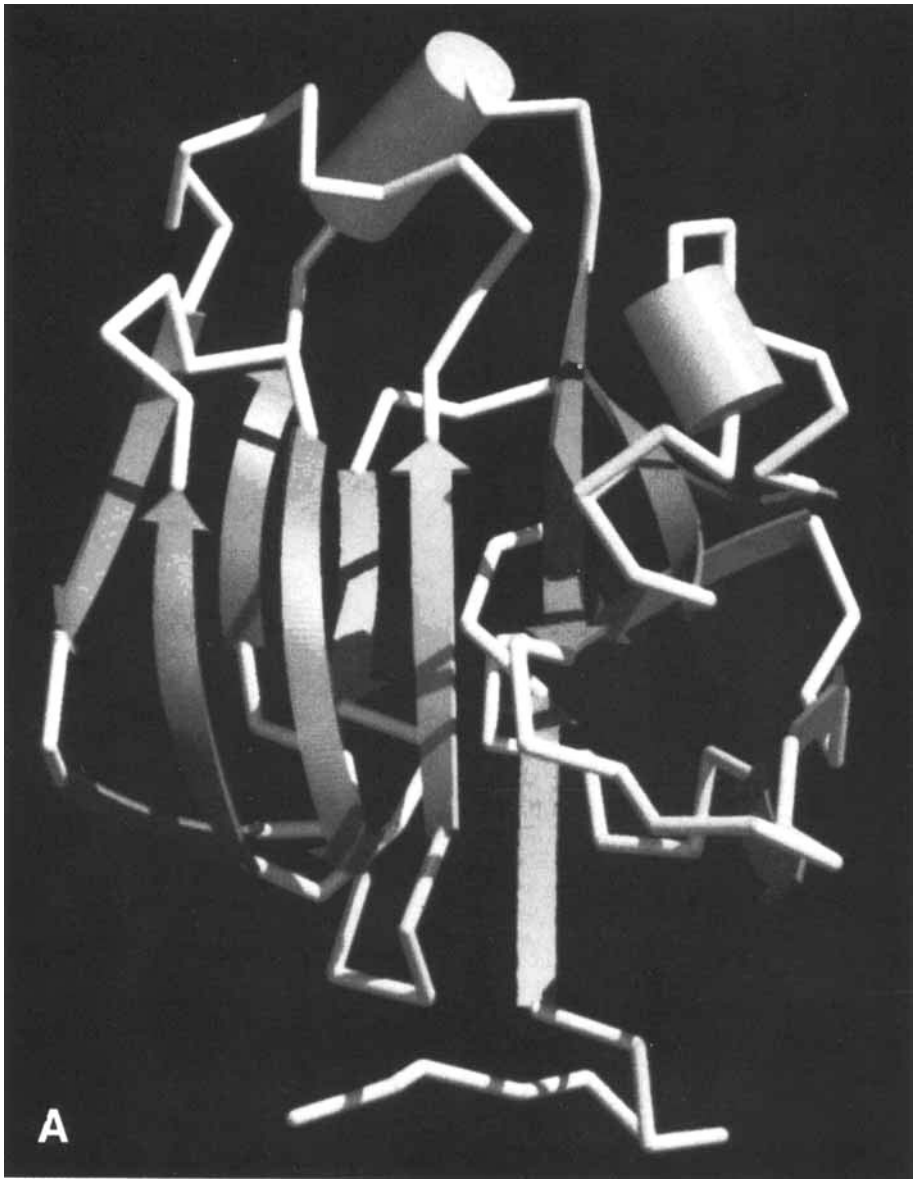


Fig. 3 (3B and legend appear on page 66)

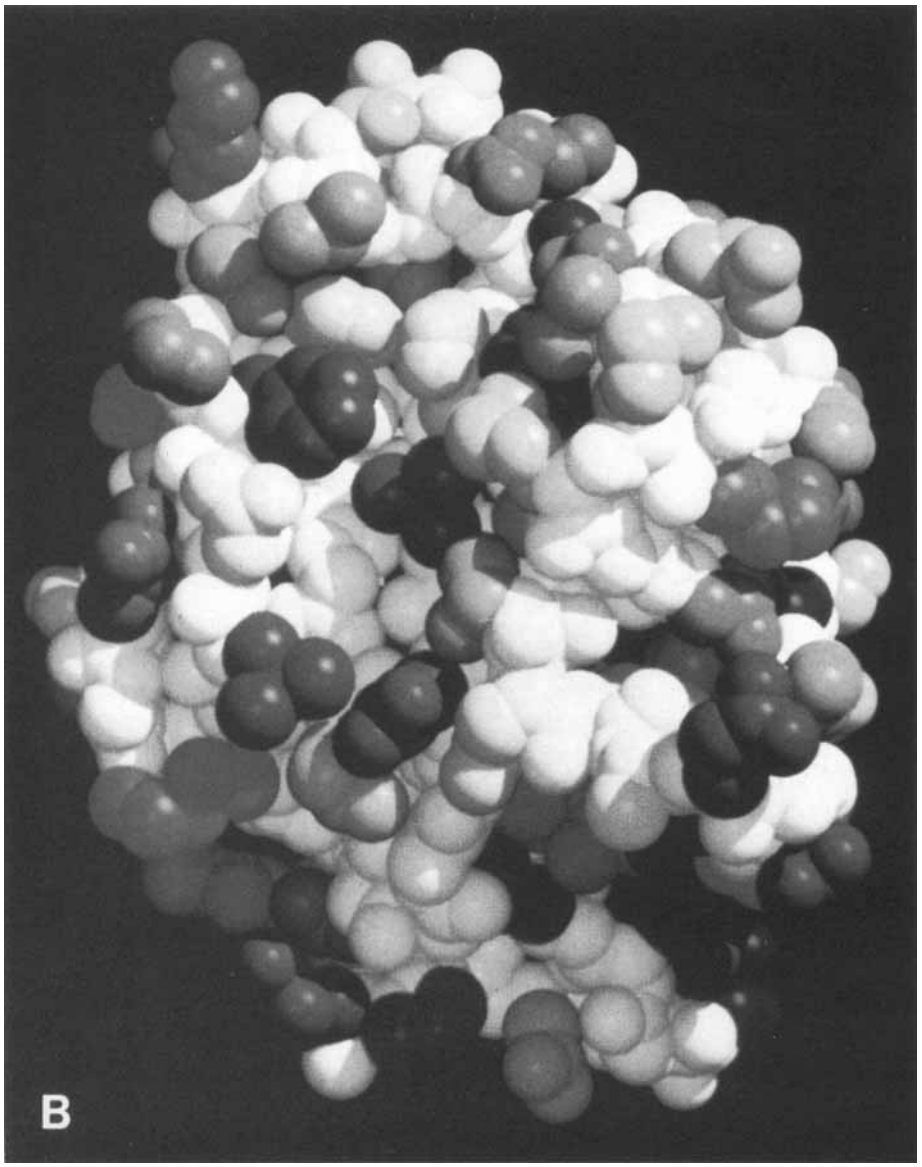


Fig. 3. The three-dimensional structure of IIA^{glc} of *B subtilis* This protein is homologous to IIA^{glc} of enteric bacteria and can replace it in the regulatory system depicted in Figure 1 The structure shown is an anti-parallel β-barrel with Greek key and jelly roll topological features **A:** The overall fold, highlighting secondary structure motifs β-strands are shown as ribbons, helices (longer than four residues) as cylinders The active-site His83 (equivalent to His 90 in the *E coli* protein) is located at the C-terminus of the central β-strand **B:** Computer-generated space-filling model of the IIA^{glc} molecule The orientation is the same as in A Two perpendicular histidyl side chains can be seen at the top third of the molecule The histidine on the right is the active-site His83, and to the left is His68 (reproduced from Liao et al , 1991, with permission)

giving rise to decreased rates of transcription of the genes within the non-PTS regulons. These two phenomena are loosely termed "catabolite repression" and "inducer exclusion."

THE INVOLVEMENT OF THE FruR PROTEIN IN TRANSCRIPTIONAL REGULATION OF CENTRAL PATHWAYS OF CARBON METABOLISM

Evidence has been presented suggesting that the fructose repressor, FruR, and possibly other proteins encoded within the fructose (*fru*) regulon of *Salmonella typhimurium* play a key role in the transcriptional regulation of central pathways of carbon source degradation and carbohydrate synthesis [Geerse et al., 1986, 1989a,b; Chin et al., 1987, 1989]. These pathways include the anaerobic glycolytic pathway (negatively controlled by FruR) and aerobic pathways of gluconeogenesis, the glyoxalate shunt, and the Krebs cycle (positively controlled by FruR) [Chin et al.,

1989]. Constituents of the electron transfer chain are also under positive transcriptional control by FruR, and *Salmonella* pathogenesis is attenuated in *fruR*::Tn10 insertion mutants [Saier and Chin, 1990]. Some evidence suggests that one of the fructose-specific PTS proteins, the diphosphoryl transfer protein (DTP) of enteric bacteria, plays a role in the FruR-mediated regulatory process. DTP is a structurally complex protein, consisting of three domains: one (at the C-terminus) resembling in sequence HPr of the PTS, a second (at the N-terminus) representing the fructose-specific Enzyme IIA, and a third (centrally located) presumably functioning as a regulatory domain because it exhibits limited sequence similarity to the consensus sequence for regulatory domains of response regulators of the so-called 2-component systems [Stock et al., 1989; Wu et al., 1990]. Exactly how this tri-domain protein participates in regulation is not known, but molecular genetic experiments aimed at answering this question are currently in progress (N.B. Vartak and M.H. Saier, Jr., unpublished experiments).

The *fruR* gene encoding the fructose repressor was independently cloned and sequenced in three laboratories [Leclerc et al., 1990; Jahreis et al., 1991; Vartak et al., 1991]. FruR proved to be homologous to a large number of DNA binding proteins, most of which are sugar-specific repressors. These proteins include the lactose (LacI), purine (PurR), cytidine (CytR), galactose (GalR), and maltose (MalI) repressors of *E. coli* (Ec), as well as a protein in *Bacillus subtilis* (Bs) (CcpA) which mediates catabolite repression of certain target operons (Fig. 4). Surprisingly the periplasmic ribose binding proteins (RbsB) of *E. coli* and *S. typhimurium* (St), constituents of the ribose transport and chemoreception systems, were also homologous to the repressor proteins. Further, the periplasmic ribose binding proteins exhibit sufficient sequence similarity to the arabinose and galactose binding proteins to suggest that these periplasmic receptors are also members of this family of homologous proteins (R. Tam and M.H. Saier, Jr., unpublished results). More detailed analyses revealed that the DNA binding proteins all have N-terminal helix-turn-helix motifs involved in DNA sequence recognition, while the periplasmic binding proteins instead possess N-terminal, hydrophobic, periplasmic-targeting (signal) sequences [Vartak et al., 1991]. The regions of these proteins exhibiting sequence similarity were the

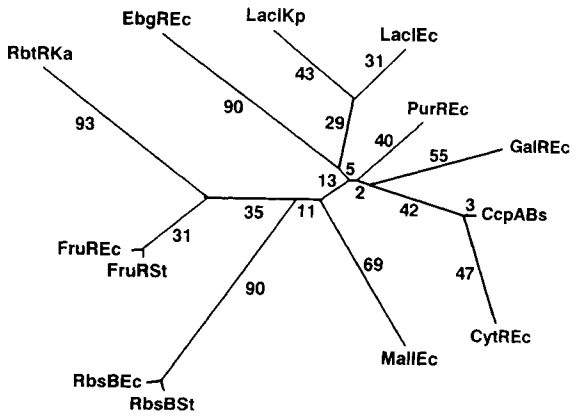


Fig. 4. Phylogenetic tree of proteins homologous to FruR. Relative evolutionary distances are provided next to the branches and were determined as described previously [Feng and Doolittle, 1990; Doolittle and Feng, 1990]. Abbreviations are provided in the text and in Vartak et al. (1991) (reproduced from Vartak et al., 1991 with permission).

C-terminal sugar binding domains. Thus, it is clear that periplasmic sugar receptors and cytoplasmic sugar repressors are derived from a common ancestor. These differing N-terminal structural features account both for their different cellular locations and their different functions.

Several genes corresponding to targets of FruR transcriptional regulation have been sequenced. These include the fructose (*fru*) operon, the *pts* operon encoding the energy coupling proteins of the PTS, Enzyme I and HPr, and the phosphofructokinase gene (*pfk*) (all negatively regulated) as well as the PEP carboxykinase (*pck*) gene, the PEP synthase (*pps*) gene, the isocitrate dehydrogenase (*icd*) gene, the *cyd* operon, encoding the multisubunit cytochrome d complex, and the *ace* operon, encoding the glyoxalate shunt enzymes (all positively regulated). The regulatory regions preceding these genes surprisingly do not show elements of striking sequence similarity (J. Reizer, A. Reizer, and M.H. Saier, Jr., unpublished), suggesting that the FruR protein does not recognize a single consensus sequence. However, upstream from the *fruB, ptsH, ppsA,* and *icd* structural genes are FruR binding sites of similar sequence (T.M. Ramseier, unpublished results), and in the *ace* operon is a region which has been shown to bind FruR with high affinity (A. Cozzone, personal communication). Further work will be required to establish the molecular mechanism with which FruR mediates transcription regulation.

PRESUMED PHYSIOLOGICAL CONSEQUENCES OF FruR-MEDIATED REGULATION

What are the physiological consequences of FruR-mediated regulation? *fruR* mutants which lack this protein show enhanced activities of rate-limiting glycolytic enzymes such as the PTS enzymes and phosphofructokinase, but decreased activities of gluconeogenic and glyoxalate shunt enzymes as well as of certain proteins required for aerobic growth (certain Krebs cycle enzymes and electron transfer carriers) [Chin et al., 1989; Saier and Chin, 1990]. Further, the effects of *fruR* mutations on gene expression are similar to those of a readily metabolizable PTS sugar such as glucose or fructose [Chin et al., 1989]. Thus, FruR may help to mediate the transition between aerobic (oxidative) and anaerobic (fermentative) growth by being responsible for the phenomenon of catabolite repression of the enzymes of aerobic metabolism. This postulate, however, is not yet fully established, and some of these genes are clearly responsive to other mechanisms of catabolite repression (i.e., the cyclic AMP-CRP-mediated mechanism). It seems likely that multiple mechanisms of catabolite repression are operative in a single bacterium such as *E. coli* [Saier, 1991].

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REFERENCES

Branden C, Tooze J (1991): "Introduction to Protein Structure." New York, London: Garland Publishing, Inc.
Chen Y, Fairbrother WJ, Wright PE: J. Cell Biochem. (this symposium.)
Chin AM, Feucht BU, Saier MH Jr (1987): J Bacteriol 169:897–899.
Chin AM, Feldheim DA, Saier MH Jr (1989): J Bacteriol 171:2424–2434.
Dean DA, Reizer J, Nikaido H, Saier MH Jr (1990): J Biol Chem 265:21005–21010.
Doolittle RF, Feng D-F (1990): Methods Enzymol 183:659–669.
Fairbrother WJ, Gippert GP, Reizer J, Saier MH Jr, Wright PE (1992): Febs Lett 296:6896–6907.
Feng D-F, Doolittle RF (1990): Methods Enzymol 183:375–387.
Geerse RH, Ruig CR, Schuitema ARJ, Postma PW (1986): Genetics 203:435–444.
Geerse RH, Izzo F, Postma PW (1989a): Mol Gen Genet 216:517–525.
Geerse RH, van der Pluijm J, Postma PW (1989b): Mol Gen Genet 218:348–352.
Jahreis K, Postma PW, Lengeler JW (1991): Mol Gen Genet 226:332–336.
Kühnau S, Reyes M, Sievertsen A, Shuman HA, Boos W (1991): J Bacteriol 173:2180–2186.
Leclerc G, Noël G, Drapeau GR (1990): J Bacteriol 172:4696–4700.
Liao D-I, Kapadia G, Reddy P, Saier MH Jr, Reizer J, Herzberg O (1991): Biochemistry 30:9583–9593.
Nelson SO, Lengeler J, Postma PW (1984): J Bacteriol 160:360–364.
Nelson SO, Schuitema ARJ, Postma PW (1986): Eur J Biochem 154:337–341.
Novotny MJ, Frederickson WL, Waygood EB, Saier MH Jr (1985): J Bacteriol 162:810–816.
Osumi T, Saier MH Jr (1982a): Ann Microbiol 133A:269–273.
Osumi T, Saier MH Jr (1982b): Proc Natl Acad Sci USA 79:1457–1461.
Reizer J, Sutrina S, Wu L-F, Deutscher J, Reddy P, Saier MH Jr (1992): J Biol Chem 267:9154–9169.
Roepe PD, Consler TG, Menezes ME, Kaback HR (1990): Res Microbiol 141:290–299.
Saier MH Jr (1977): Bacteriol Revs 41:856–871.
Saier MH Jr (1989a): Microbiol Revs 53:109–120.
Saier MH Jr (1989b): Res Microbiol 140:349–352.
Saier MH Jr (1991): The New Biologist 3:1137–1147.
Saier MH Jr, Chin AM (1990): Bacterial Energetics. Academic Press, London, New York, pp. 273–299.
Saier MH Jr, Reizer J (1990): Res Microbiol 141:1033–1038.
Saier MH Jr, Reizer J (1992): J Bacteriol 174:1433–1438.
Saier MH Jr, Wu L-F, Reizer J (1990): Trends Biochem Sci 178:391–395.
Stock JB, Ninfa AJ, Stock AM (1989): Microbiol Rev 53:450–490.
Sutrina SL, Reddy P, Saier MH Jr, Reizer J (1990): J Biol Chem 265:18581–18589.
Vartak NB, Reizer J, Reizer A, Gripp JT, Groisman EA, Wu L-F, Tomich JM, Saier MH Jr (1991): Res Microbiol 142:951–963.
Wilson TH, Yunker PL, Hansen CL (1990): Biochim Biophys Acta 1029:113–116.
Worthylake D, Meadow ND, Roseman S, Liao D-I, Herzberg O, Remington SJ (1991): Proc Natl Acad Sci USA 88: 10382–10386.
Wu L-F, Tomich JM, Saier MH Jr (1990): J Mol Biol 213:687–703.
Zeng GQ, De Reuse H, Danchin A (1992): Res Microbiol 143:251–261.