Minireview

Signal Transduction by the Bacterial Phosphotransferase System

DIAUXIE AND THE err GENE (J. MONOD REVISITED)*

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The phosphoenolpyruvate:glycose phosphotransferase system (PTS) is an array of cytoplasmic and membrane proteins with diverse physiological roles in the bacterial cell. The best defined function of the PTS is the phosphorylation of sugar substrates concomitant with their transport across the cytoplasmic membrane, a remarkably efficient group translocation process for capturing nutrients from the environment. The metabolic equivalent of one ATP (i.e., P-enolpyruvate) is used to simultaneously transport, trap, and incorporate one molecule of sugar into its catabolic pathway.

The PTS is also essential for many other processes. For example, it is required for chemotaxis toward its substrates, and it regulates the transcription of operons necessary for the catabolism of certain non-PTS sugars, the subject of this review. Since the PTS is ubiquitous in the most widespread of marine bacterial genera (Vibrio), it plays a key role in the carbon and nitrogen cycles in the marine environment (3); it is also required for processes as different as those that produce dental caries, cheese, and soy sauce (3, 4). These diverse functions have led to intensive study of the PTS at the biochemical and genetic levels, and it is the subject of many recent reviews (for example see Refs. 3–8). The present brief description is written principally for the general reader and emphasizes only the signal-transducing function of the PTS in “diauxie.”

Diauxie Growth

Toward the end of the last century it was observed that bacteria grown on glucose produce barely detectable levels of many enzymes compared with the same cells grown on other carbon sources. This inexplicable result, designated the “gluconae-trophism” during the 1940s. Monad’s approach (9) was to determine the metabolic equivalent of one ATP (i.e., P-enolpyruvate) is used to simultaneously transport, trap, and incorporate one molecule of sugar into its catabolic pathway.

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Diauxie Growth

Toward the end of the last century it was observed that bacteria grown on glucose produce barely detectable levels of many enzymes compared with the same cells grown on other carbon sources. This inexplicable result, designated the “glucose effect,” was quantitatively studied by Monod and others during the 1940s. Monod’s approach (9) was to determine the growth rates of Bacillus subtilis, Escherichia coli, and Salmonella typhimurium in chemically defined media containing two sugars. Results typical of a large number with a variety of sugars are shown in Fig. 1. With certain pairs of sugars, a single exponential growth curve was obtained, whereas other pairs of sugars gave a biphasic (“diauxic”) growth curve.

The diauxic curve consists of three stages. First, the cells grow exponentially while they completely consume a preferred sugar such as glucose. They then enter a lag phase, and the third stage consists of exponential growth on the second sugar. Later work on lactose utilization showed that transcription of the lac operon is repressed during growth of the cells on glucose and begins during the lag phase. These results ultimately led to the classic work of Jacob and Monod on gene regulation.

Monod made two additional observations that require emphasis here. First, diauxie is a quantititative phenomenon. For instance, a large diauxic effect, much greater than that shown in Fig. 1, was observed when the xylose/glucose ratio was 1:0; as the ratio was increased, the diauxic effect became less and less until it disappeared. Second, bacteria have memory (our words), as shown in Fig. 1 (right panel). That is, the degree of diauxie can depend on the medium used for growth of the inoculum. Monod did not comment on the strange fact that glucose promotes the maximum diauxic effect while maltose does not, even though the disaccharide is degraded to glucose and glucose-P inside the cell. How do the enteric bacteria sense external glucose and differentiate it from internally generated glucose and glucose metabolites? What is the signal-transducing mechanism?

The Bacterial Phosphotransferase System

The PTS was discovered as a glucose/mannose-phosphorylating system with several unique features (10). The phosphoryl donor was P-enolpyruvate, three protein fractions (Enzyme I, HPr, and Enzyme II) were required, phosphohistidyl-HPPr was an intermediate in the sequence, and Enzyme II, the sugar-specific component of the system, was membrane-bound. The phosphotransfer reaction pathway in Fig. 2 was subsequently established with purified proteins. The PTS exhibits great diversity of structure and complexity of interactions with other metabolic pathways. Recent work has identified over two dozen sugar-specific proteins (Enzyme II complexes) that are quite variable in their chemical and physical properties (3). Other proteins not in the PTS pathway, such as acetate kinase, also interact with the PTS.

Variation in the PTS—Variation is an underlying motif of the PTS. From the perspective of understanding how the systems work, the variations of major interest are the number and types of proteins in the phosphotransfer sequences. The phosphotransfer from P-enolpyruvate to sugar may require as few as two protein types as many as five (3) (Fig. 2). This kind of variation exists within a single organism. In E. coli, for instance, the systems for mannitol and G/NeAc require three proteins each, the glucose-specific system requires four, while the relatively nonspecific mannose/glucose system requires five. Furthermore, some phosphotransfer sequences utilize chimeric proteins that combine functions usually fulfilled by two independent PTS proteins, such as Enzyme I and HPr in one case and a combination of HPr and a Factor III-like fructose-specific protein in another.

The sugar-specific Enzyme II complexes, which transfer the phosphoryl group from P-HPPr to sugar, are currently the focus of major interest. As already indicated, they consist of

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The abbreviations used are: PTS, phosphoenolpyruvate:glycose phosphotransferase system; Mtl, mannitol; P-enolpyruvate, phosphoenolpyruvate.

2 We were unable to include 33 references to primary publications because of limitations of space; these references are found in the cited reviews and are also available on request.
group is transferred reversibly to the active site histidine of phosphate has been reported in IImt. Acetate kinase is not phosphoryl group is linked to N' or N3 of the imidazole ring of histidine moieties in PTS proteins; a cysteine-linked thio-

one to three polypeptide chains (Fig. 2). Many of the genes have been cloned and sequenced, and important evolutionary implications have been drawn from similarities in the sequences; these are considered below.

There are other variations in the phosphotransfer steps (3). (i) Exceptions are being reported to the general rule that the phosphoryl group is linked to N1 or N3 of the imidazole ring of histidine moieties in PTS proteins; a cysteine-linked thiol-phosphate has been reported in IIMod. Acetate kinase is not strictly part of the PTS, but it is autophosphorylated by ATP, GTP, or acetyl-P at a glutamate residue, and the phosphoryl group is transferred reversibly to the active site histidine of

Enzyme I. Indeed, the kinase and ATP (or GTP) can replace P-enolpyruvate for sugar phosphorylation by the PTS in vitro. A serine residue in HPr of Gram-positive bacteria undergoes a phosphorylation that appears to be regulatory. (ii) The sugar receptor IIB proteins may or may not be phosphorylated.

IIBMod of Fig. 2 may be phosphorylated as part of the reaction sequence, whereas there is no detectable phosphorylation of IIBMas or IIBMen. (iii) Until recently, only intermolecular phosphotransfers were recognized, but there is evidence of intramolecular transfers. A phosphotransfer from one histidine to another apparently occurs within the IIIMod protein of Fig. 2. Enzymes II consisting of single polypeptide chains contain domains at their N termini that are typical of IIB-type proteins and domains at their C termini analogous to the III-type proteins. The latter apparently accepts the phosphoryl group from phospho-IIIg, which is then presumably transferred intramolecularly to a histidine residue in an N-terminal domain and finally to the sugar.

Why the Complexity? Why the Variations?—Variation in the proteins may have resulted from the evolutionary divergence of a single primordial gene or by the convergent evolution of distinctly different, independent genes.

Theoretically the sugar transport/phosphorylation function of the PTS could be catalyzed by an ATP-dependent membrane-bound sugar kinase; ATP would provide more than enough energy for the translocation, and specificity would be inherent in the kinase. So why is the PTS so complex? And why are there so many variations? We suggest at least three answers to these questions.

(i) Complexity may facilitate evolutionary flexibility. This concept may apply to non-PTS as well as to PTS proteins. For example, lactose is a PTS sugar in many Gram-positive bacteria (4, 8) but is transported by proton motive force-driven permeases in E. coli and in the Gram-positive organisms Streptococcus thermophilus and Lactobacillus bulgaricus. The genes encoding the lactose permeases of the latter two species have recently been sequenced (11). There is a striking similarity to E. coli IIIMod especially in the region corresponding to the active site of the latter (Fig. 3). These and other data (5) suggest that some PTS genes may have evolved into genes encoding permeases that function by mechanisms other than group translocation.

Fig. 3. Deduced amino acid sequences containing the active site histidine residues (*) of E. coli and S. typhimurium IIIMod and proposed regulatory sites in two proton motive force-driven lactose permeases from S. thermophilus and L. bulgaricus (11). The number preceding the sequence is the position of the first residue given.
The PTS and Diauxie

PTS sugars are preferred by cells that exhibit diauxie, and when its substrates are present in the medium, the PTS represses induction of many non-PTS operons. By what mechanisms? While we suppose that PTS-mediated repression involves multiple mechanisms, only two have thus far been identified and partially characterized in E. coli and S. typhimurium. The crl gene and IIIFr.—The work of Monod was followed by extensive studies on the glucose effect, particularly on the lactose operon in E. coli (12, 13). Three key observations were: glucose inhibited the uptake of the inducing β-galactoside (designated “inducer exclusion”); cAMP was necessary for induction; glucose greatly decreased the level of intracellular cAMP. Thus, glucose (and other PTS sugars) simultaneously lowered the intracellular concentrations of the inducer and cAMP, the two ligands required for induction. Several catalytic systems in E. coli and S. typhimurium (the operons for glycerol, lactose, maltose, and melibiose) are sensitive to repression by PTS sugars such as glucose, methyl α-glucoside, and mannitol (3, 9). PTS sugars inhibited inducer uptake even though each of the four perpanes functions by a different mechanism: glycerol by facilitated diffusion, melibiose by Na+ symport, lactose by H+ symport, and maltose by ATP hydrolysis.

The isolation of Crl- mutants (14) provided an important clue to understanding PTS-mediated repression; a single mutation (crr, "carbohydrate repression resistance") reversed repression of the four catalytic/transport systems simultaneously. What was the product of the crr locus, and what did it do? The only detectable changes in extracts of the mutants were lowered levels of IIIFr (0-30% of wild type) activity. Because the mutation had a pleiotropic phenotypic effect, this meant either that crr encoded IIIFr or that it encoded a protein that regulated the expression of activity of many operons including the IIIFr gene. Biochemical and genetic methods were used to determine the relationship between the crr gene and IIIFr, and transposon mutagenesis ultimately provided unequivocal evidence that crr encodes IIIFr (3, 6-13).

Signal Transduction by the PTS

Regulation by IIIFr.—The observation that Crr- mutants contained low levels of IIIFr led to hypotheses explaining how the PTS regulated non-PTS permeases (15) and adenylate cyclase, long before it was actually known that crr encoded IIIFr. The model shown in Fig. 4 illustrates the original concept: IIIFr combines with and inhibits the sensitive permeases, whereas phospho-IIIFr is either inactive or is a positive effector. Experiments with intact cells, membrane vesicles, and reconstituted systems support the model (3, 6, 7). While all results are consistent with the proposed scheme, we emphasize that the model may be too simple. In vitro reconstitution studies with purified IIIFr and the lactose permease in synthetic membrane vesicles gave substantially smaller effects than obtained with IIIFr and natural membrane vesicles. Either the reconstitution conditions are not optimal or there is a missing component(s).

The interaction between adenylate cyclase and the PTS has also been extensively studied (12, 13, 17). The current hypothesis for this regulation is the reciprocal of the model shown in Fig. 4: that is, phospho-IIIFr is an activator of adenylate cyclase while IIIFr is either inactive or is an inhibitor. Experiments with toluene-permeabilized cells gave results consistent with this idea. In vitro reconstitution experiments were again only partially successful, although it was shown that the regulation requires other PTS proteins and perhaps other factors as well. Therefore, we believe that the model in Fig. 4 and the reciprocal model for regulating adenylate cyclase are correct but may be incomplete. Further work is surely required to fully explain the in vivo effects.

The Phospho-IIIFr/IIIFr Ratio.—In both models, the ratio of phospho-IIIFr to IIIFr is the key element in regulation, providing the mechanism for signal transduction by the PTS. When extracellular glucose or methyl α-glucoside interacts with IIIFr, phospho-IIIFr is converted to IIIFr. The decrease in intracellular P-enolpyruvate/pyruvate, and sugar transport is stringently controlled. Would it not be important, or even essential, to couple regulation of PTS-mediated repression; a single mutation CFF, the two ligands required for induction. Several catalytic systems in E. coli and S. typhimurium. The operons for glucose, methyl α-glucoside, and mannitol (3, 9). PTS sugars inhibited inducer uptake even though each of the four perpanes functions by a different mechanism: glycerol by facilitated diffusion, melibiose by Na+ symport, lactose by H+ symport, and maltose by ATP hydrolysis.

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in the phospho-III\textsuperscript{Glc}/III\textsuperscript{Glc} ratio inhibits the non-PTS permeases and adenylate cyclase simultaneously, resulting in repression of transcription of the operon (12, 13). With the exception of the final phosphotransfer to the sugar, the sequential transfer reactions in Fig. 2 are readily reversible (3, 7). Therefore, any external PTS sugar should decrease the pool size of the phospho-PTS proteins, thereby decreasing the ratio of phospho-III\textsuperscript{Glc} to III\textsuperscript{Glc} and giving repression. Indeed, in Monod’s experiments (9), mannitol could substitute for glucose. However, diauxie was not observed with the PTS sugars mannose, fructose, and sorbitol. Why not?

The explanation lies in Monod’s data; diauxie is a quantitative phenomenon. This idea is also inherent in the model of Fig. 4. III\textsuperscript{Glc} and/or phospho-III\textsuperscript{Glc} interact stoichiometrically, not catalytically, with at least the proteins shown in Fig. 5. In other words, the critical factors that determine diauxie are the absolute concentrations of the proteins, the ratios of the inhibitable non-PTS proteins and III\textsuperscript{Glc}, the ratio of phospho-III\textsuperscript{Glc} to III\textsuperscript{Glc}, and the binding constants of the different interacting species. Furthermore, the permeases and glycerol kinase are not completely inhibited even in the presence of “saturating” levels of the PTS proteins; adenylate cyclase, however, is inhibited by more than 90% in the presence of PTS sugars. Finally, diauxie will or will not result, depending upon the kinetics of the interactions. That is, the steady state ratio of phospho-III\textsuperscript{Glc} to III\textsuperscript{Glc} may vary with the PTS sugar since the sugars are utilized at different rates (6, 12, 13, 17). This concept would also explain why internally generated glucose (from maltose) does not have the same effect as external glucose in Fig. 1.

We can now turn back to the problem of “bacterial memory.” The conditions of growth of the inoculum determine the levels of the interacting species at the beginning of the experiment. If cells are preinduced to high levels of non-PTS permeases and lowered levels of the PTS regulatory proteins, they will be resistant to PTS-mediated repression (13). If the converse is true, they will be hypersensitive to the phenomenon.

**Perspectives**

Assuming that the simple models presented above are correct, an important goal will be to understand the many functions of III\textsuperscript{Glc}. One approach is to construct site-directed mutations, a method that has already given unexpected results (18), as shown in Fig. 6. Replacement of the active site His-90 gave a mutant III\textsuperscript{Glc} that was inactive in methyl \(\alpha\)-glucoside phosphorylation (as expected), but surprisingly, this mutation did not alter the Crr phenotype. Even more unexpectedly, replacement of His-75 gave a mutant III\textsuperscript{Glc} that accepted the phosphoryl group (at His-90) but did not act as a phosphoryl donor. This mutation produced a Crr\(^{-}\) phenotype with some non-PTS sugars but not with others.

This brief review shows that we are in the qualitative or descriptive phase of work on the PTS and associated proteins and genes; present studies are focused on identifying all of the interacting components. In the next decades, however, we can expect that we will see more and more effort applied to quantitative characterization of these molecular interactions. The uptake of the PTS and non-PTS sugars appears to be the rate-limiting step in the growth of the cells, and this being the case, determining the relevant binding constants, concentrations, steady state levels, kinetic constants, etc. may finally permit us to predict the curves shown in Fig. 1 and in J. Monod’s Ph.D. thesis.

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