BACTERIOLOGICAL REVIEWS, Dec. 1977, p. 856-871 Vol. 41, No. 4
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Bacterial Phosphoenolpyruvate:Sugar Phosphotransferase Systems: Structural, Functional, and Evolutionary Interrelationships

MILTON H. SAIER, JR.

Department of Biology, The John Muir College, The University of California, San Diego, La Jolla, California 92093

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What web is this
Of will be, is, and was?
-Jorge Luis Borges

INTRODUCTION

Many procaryotic species possess a complex phosphoenolpyruvate (PEP):sugar phosphotransferase system (PTS). In *Escherichia coli* and *Salmonella typhimurium,* the organisms for which the most detailed information about the PTS is available, the system catalyzes the concomitant transport and phosphorylation of several sugars. It also functions in a regulatory capacity, controlling the rates of cellular carbon and energy metabolism. Recent studies have led to the suggestion that the complexity of the PTS arose through evolutionary history in order to allow greater control over those bacterial physiological processes that are directly or indirectly involved in carbon acquisition. Yet, in spite of the importance of this postulate, systematic efforts to evaluate the possibility have been lacking. A few comparative studies have been conducted to provide information about the evolutionary origins of the PTS, but the results of these investigations have not been systematically analyzed. In this review an attempt is therefore made to assess the field from several related standpoints. First, the primary and secondary functions of the PTS are discussed to gain an understanding of the evolutionary benefits that might be conferred upon a microorganism by the possession of a complex PTS. Second, an attempt is made to ascertain the basis for the fact that some procaryotic species possess the PEP-dependent, sugar-phosphorylating capacity whereas other bacteria appear to lack this capacity altogether. Third, less complex PTSs in selected bacterial genera are examined to gain information concerning the end products of presumably divergent evolutionary pathways. The data discussed permit rationalization of several observations regarding a number of structural and functional features of the PTS. They also allow postulation of pathways by which simple primordial enzymes may have evolved into multifunctional, multicomponent transmembrane transport systems. The postulates and interpretations advanced in this review emphasize the value of the comparative approach and the applicability of the unity principle to molecular evolution (35).

(This review was presented in part at the 1976 Annual Meeting of the West Coast Bacterial Physiologists in Asilomar, Calif., and was prepared for publication at the instigation of Roger Y. Stanier.)

TRANSPORT MECHANISMS IN BACTERIA

Studies carried out in numerous laboratories over the past 20 years have established that simple carbohydrates enter bacterial cells by several distinct mechanisms (35). These mechanisms have been denoted facilitated diffusion, primary and secondary active transport, and group translocation (35). The simplest of these transport processes, facilitated diffusion, merely

involves the translocation of a solute molecule down its concentration gradient from one side of the membrane to the other. The process is mediated by a membrane protein or permease, which exhibits stereospecificity towards its substrates. Because the translocation step is not coupled to metabolic energy, the solute cannot be accumulated within the cell against a concentration gradient.

Active transport and group translocation resemble facilitated diffusion in that these processes require the participation of specific membrane-associated transport proteins, which catalyze stereospecific vectorial reactions. They differ from facilitated diffusion in the one respect that solute translocation is coupled to metabolic energy. Consequently, solute can be accumulated intracellularly against a concentration gradient. The fundamental difference between group translocation and active transport deals with the nature of the product released into the cytoplasm. In the case of active transport, the molecule that appears in the cytoplasm is the same chemical species that was removed from the extracellular medium. In referring to primary active transport, a chemical or electrical source of energy drives the uptake process; in secondary active transport, ion fluxes are coupled to ionic electrochemical gradients, which in turn are generated by primary active transport. In contrast, group translocation involves modification of the solute during its passage through the membrane; the solute is changed chemically during transmembrane transport. It is this last process, group translocation, that is thought to be catalyzed by the PTS. The solute molecule taken up from the external medium is a sugar, and the derivatized form of that sugar which enters the cytoplasmic space is the corresponding sugar phosphate. The sugar becomes phosphorylated in transit, during passage through the plasma membrane. Pertinent information regarding this group translocation process is discussed in the next section.

ACTIVE AND EXCHANGE GROUP TRANSLOCATION OF SUGARS CATALYZED BY THE BACTERIAL PTS

The overall chemical reaction catalyzed by the PTS involves transfer of the phosphoryl moiety of PEP to sugar in the presence of the enzyme components of the PTS and Mg^{2+}: PEP + sugar $\xrightarrow{\text{PTS} + Mg^{2+}}$ sugar-P + pyruvate. The products of the reaction are sugar phosphate and pyruvate. The reaction resembles that catalyzed by a simple adenosine 5'-triphosphate (ATP)-dependent sugar kinase except that the phosphoryl donor is PEP instead of ATP. One might therefore expect that a single polypeptide chain would catalyze this reaction, but extensive enzymological analyses have revealed that this is not the case. Phosphoryl transfer from PEP to sugar requires the intermediate participation of several catalytic proteins (22). Two general, non-sugar-specific proteins of the PTS, enzyme I and the low-molecular-weight, heat-stable protein HPr, initiate a sequence of phosphoryl transfer reactions. These two cytoplasmic proteins function in the transport and phosphorylation of all of the sugar substrates of the system. The sugar-specific proteins of the system, the components of the enzyme II complexes, are the enzymes II and III (16, 17, 22, 35). The enzymes II are always found as integral components of the plasma membrane in association with phospholipid and divalent cations, which are essential for their activity (16, 17). In contrast, the enzymes III may be either membrane associated as components of the enzyme II complexes or released from disrupted cells as fully soluble proteins. In the former case, appropriate treatment of the membrane releases these peripheral membrane constituents (39) as water-soluble enzymes (17).

Since several sugars are phosphorylated via the PTS, and since each enzyme II complex phosphorylates and transports only one or a few such sugars, it is apparent that the bacterial cell must possess several pairs of the sugar-specific proteins. Thus, one pair of enzymes II and III catalyzes the phosphorylation of glucose, and another pair of these enzymes is involved in the phosphorylation of fructose, whereas yet a third enzyme II complex functions in the phosphorylation of mannitol. In fact, since the PTS in *E. coli* phosphorylates about 10 different sugars, each of which is recognized by a different enzyme II complex, there may exist in the bacterial cell about 10 pairs of sugar-specific, membrane-associated proteins. Thus, the complexity of the PTS becomes apparent. The entire system consists of about two dozen proteins: two general energy-coupling enzymes and about 10 pairs of sugar-specific proteins.

Exactly how these proteins are thought to function in the transport and phosphorylation of sugars is shown schematically in Fig. 1. This figure illustrates the nature of the phosphate transfer chain of the PTS, elucidated by the elegant biochemical analyses of Roseman and his collaborators (16, 17, 22, 37). Phosphate is not transferred directly from PEP to sugar but is sequentially transferred down the phosphate transfer chain of the PTS, first, from PEP to enzyme I, and then to HPr. In both of these general "energy-coupling" enzymes of the PTS, the phosphoryl moiety is covalently linked to

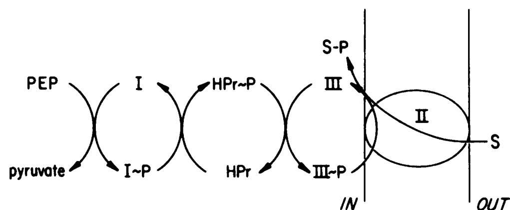
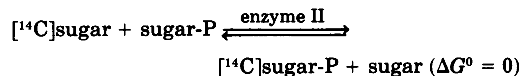


FIG. 1. *Phosphate transfer chain of the PTS of many eubacterial species. I, II, and III represent enzymes I, II, and III, respectively.*

histidyl residues in the proteins. The third step in the sequence of phosphoryl transfer reactions involves transfer of the phosphoryl moiety of phospho-HPr, from this "general" protein of the system to a sugar-specific component, an enzyme III. The phosphoryl moiety in one of these oligomeric proteins may be linked to either a glutamyl or a histidyl residue in the protein, depending on the enzyme III under examination. In all cases the phosphoryl proteins formed are of high energy with free energies of hydrolysis nearly equivalent to that found for the initial phosphoryl donor, PEP. The fourth and last step in this sequence of reactions results in sugar phosphorylation. The phosphoryl moiety of phospho-enzyme III is transferred to sugar in a process that requires the participation of the membrane-associated enzyme II complex. The reaction is apparently accompanied by transmembrane sugar translocation.

Since the enzyme II is the constituent of the PTS that binds the sugar substrate (37) and presumably functions as the permease for sugar translocation (29), this protein is the one that is of greatest interest in terms of the transport mechanism. Ironically, it is the PTS constituent about which we know the least. None of the enzymes II have been purified to homogeneity or studied from a physicochemical standpoint, nor is information available regarding the mechanism by which the enzymes II mediate transmembrane sugar translocation. Accordingly, most of the available information concerns the energy-coupling mechanism of the group translocation process and not the nature of the transport step.

In this regard, it is of interest to note that each of the enzyme II complexes of the PTS catalyzes a chemical reaction in the absence of the soluble constituents of the system (26a, 29, 29a). This reaction is as follows:

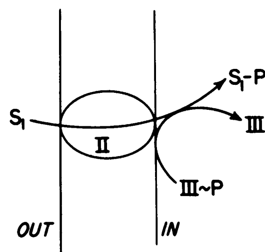


$$[^{14}C]\text{sugar} + \text{sugar-P} \xrightleftharpoons{\text{enzyme II}} [^{14}C]\text{sugar-P} + \text{sugar} \quad (\Delta G^0 = 0)$$

The reaction is a simple phosphoryl exchange reaction which can occur between a sugar phosphate and the corresponding (or a structurally related) free sugar. It is a sugar phosphate:sugar transphosphorylation reaction, which can be thought of as a "partial reaction." Although the process has been demonstrated in whole cells, its physiological significance is uncertain.

Interest in this enzyme II-catalyzed reaction stems from the observation that in intact bacterial cells and in membrane vesicles, phosphoryl exchange appears to occur in a vectorial fashion. Thus, an enzyme II complex apparently catalyzes two chemical reactions, both of which appear to be coupled to transmembrane sugar translocation. These processes, termed active group translocation and exchange group translocation, are illustrated in Fig. 2. Active group translocation is the process discussed above in which sugar is transported and phosphorylated in a coupled process requiring transfer of the phosphoryl moiety, originally derived from PEP, from phospho-enzyme III to sugar. Exchange group translocation is a process in which extracellular sugar is brought into the cell and phos-

A. Active Group Translocation (PTS)



B. Exchange Group Translocation (PTS)

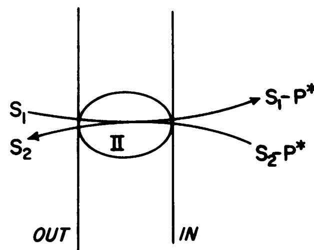


FIG. 2. *Diagrammatic representation of the two vectorial reactions thought to be catalyzed by an enzyme II complex of the PTS. (A) Active group translocation where the phosphoryl moiety of enzyme III-P is initially derived from intracellular PEP. (B) Process of exchange group translocation where the phosphoryl group of intracellular sugar-P is transferred to an incoming sugar.*

phorylated at the expense of intracellular sugar phosphate. $Sugar_1$, initially present in the extracellular fluid, traverses the membrane and is phosphorylated in a process that requires direct donation of the phosphoryl group of an intracellular sugar phosphate, $sugar_2$-P. At the same time, the sugar moiety of $sugar_2$-P is thought to be expelled from the cell. Thus, while $sugar_1$ enters the cell and is phosphorylated, $sugar_2$-P is dephosphorylated, and the $sugar_2$ moiety appears in the extracellular medium. Since both substrates are modified during transport, we refer to the process as exchange group translocation. Available evidence indicates that the mechanism requires simultaneous binding of both substrates to the surface of the enzyme and may not require phosphorylation of the enzyme II protein (A. W. Rephaeli and M. H. Saier, Jr., unpublished data). The process may occur by a channel mechanism involving an oligomeric enzyme II complex (39).

PHYSIOLOGICAL FUNCTIONS OF THE PTS

Of considerble interest to the bacterial physiologist is the spectrum of carbohydrates which serve as substrates for PTS-mediated group translocation. Table 1 summarizes some of the pertinent information for *E. coli* and *S. typhimurium*. The carbohydrates listed in the left-hand column are substrates of the PTS, termed PTS sugars, whereas those on the right, non-PTS sugars, are transported by alternative mechanisms. PTS sugars fall into two main groups, the hexitols and the hexoses. Included in the first group are mannitol, glucitol, and galactitol. Each of these substrates is phosphorylated by a distinct membrane-associated enzyme II complex. In none of the phosphoryl transfer reactions catalyzed by these enzyme II complexes has a requirement for a soluble (or peripheral membrane-associated) enzyme III been demonstrated. Thus, hexitol phosphorylation may involve direct transfer of the phosphoryl moiety from phospho-HPr to sugar.

TABLE 1. *Sugar substrate specificities of transport systems in E. coli and S. typhimurium*

Enzymes II of the PTS	Non-PTS permeases
(1) Mannitol	(1) Glycerol
(2) Sorbitol	(2) Maltose
(3) Galactitol	(3) Melibiose
(4) Lactose	(4) Lactose
(4) Glucose (methyl α-glucoside)	(5) Galactose
(5) Glucose-mannose (2-deoxyglucose)	(6) Hexose 6-phosphates
(6) Fructose	(7) Pentoses
(7) *N*-acetylglucosamine	

The second class of PTS sugars, the hexoses, includes glucose, mannose, fructose, and *N*-acetylglucosamine. Each of these substrates is similarly translocated by a distinct enzyme II complex in the membrane. The enzyme II^{glc} acts upon glucose and the non-metabolizable glucose analog, methyl α-glucoside, whereas the enzyme II^{man} exhibits broad substrate specificity, acting on glucose, mannose, 2-deoxyglucose, glucosamine, and fructose with decreasing affinity in that order (21, 22, 26a). In contrast, the enzyme II^{fru} exhibits a high degree of substrate specificity, phosphorylating only fructose to yield fructose 1-phosphate (22, 41).

Carbohydrates that enter the cell by alternative mechanisms include glycerol, which appears to be transported by facilitated diffusion (21), and the disaccharides maltose, melibiose, and lactose, which traverse the membrane by active transport processes (Table 1). Although all three disaccharides are accumulated intracellularly in an unaltered form, the energy-coupling mechanisms, which allow for substrate accumulation, appear to differ. For example, maltose enters the cell by a process that requires the participation of a periplasmic maltose-binding protein and may depend on chemical energy such as ATP. In contrast, the transport of the α- and β-galactosides, melibiose and lactose, does not involve a periplasmic binding protein and appears to depend only on transmembrane ion gradients (5). Whereas the melibiose permease appears to function by a Na^+ co-transport mechanism (40), lactose uptake appears to be coupled to proton influx (42). Thus, active transport of sugars can occur by any one of several mechanisms apparently involving the participation of chemical energy such as ATP and/or chemiosmotic energy in the form of the electrochemical gradients of specific ionic species.

Recent studies have shown that the sugar substrates of the PTS strongly inhibit the uptake of non-PTS carbohydrates in several gram-negative bacterial species. Comparable reciprocal effects are not observed (15, 28, 31, 35, 43). In other words, the PTS appears to regulate the activities of non-PTS sugar permeases, although the non-PTS permeases do not exert similar inhibitory effects on the uptake of PTS sugars. This unidirectional regulation allows the bacterium to select preferred carbon sources when more than one is present in the extracellular medium. These regulatory interactions create a hierarchy of selected substrates where PTS sugars are taken up preferentially to non-PTS carbohydrates. The PTS appears to dominate, or rule over, the activities of other permeases (28).

From these considerations it becomes appar-

ent that the PTS functions in more than a single capacity: it not only catalyzes uptake of its own sugar substrates, but it also regulates the uptake of other carbohydrates. And yet, this picture is incomplete. The known physiological functions of the PTS fall into two main categories: (i) the acquisition of carbon and energy sources (the sugar substrates of the system; this is the primary function), which can be subdivided into chemoreception, transmembrane sugar transport, sugar phosphorylation, and autoinduction of PTS proteins; and (ii) the regulation of the acquisition of other carbon sources (non-PTS carbohydrates; this is the secondary function), which can be subdivided into the regulation of flagellar synthesis, transmembrane sugar transport, cyclic adenosine 3′,5′-monophosphate (AMP) synthesis, and catabolic enzyme synthesis.

Adler and Epstein have shown that the proteins of the PTS function as a chemoreception system: they allow the bacterium to recognize sugar substrates of the PTS in the extracellular environment and to swim up concentration gradients of these compounds (1). As a result, the bacterium seeks optimal concentrations of readily utilizable extracellular sources of carbon and energy. The second and third primary functions of the PTS listed above have already been discussed: the PTS transports and phosphorylates its sugar substrates, thereby bringing these metabolites into the cytoplasm and initiating their catabolism. It is possible that chemoreception is coupled in some way to transmembrane sugar translocation and that all of the primary functions of the PTS are mechanistically related. A final primary function of the PTS involves the regulation of the synthesis of its own protein constituents. Thus, the PTS may exhibit the phenomenon of autogenous induction (6). That is, the induced synthesis of enzyme I and HPr by a sugar substrate of the PTS depends on the activity of the enzyme II complex which exhibits specificity toward that sugar as well as on their own activities. Moreover, the induced syntheses of some of the enzyme II complexes are reliant on the activities of enzyme I and HPr. In some cases, induced enzyme synthesis also appears to require the participation of extracellular rather than intracellular sugar. Induction of enzyme I and HPr, for example, occurs only when extracellular PTS sugars are present. Non-PTS sugars are ineffective even though their metabolism may generate intracellular PTS sugars or sugar phosphates (see below). A novel mechanism of transcriptional regulation may be involved.

The known secondary, regulatory functions of the PTS are listed above. One interesting target of regulation is the bacterial flagellum. Recent unpublished evidence suggests that the PTS not only regulates synthesis of the bacterial flagellar proteins, but also controls the expression of motility (R. C. Tuttle and M. H. Saier, Jr., unpublished data). Mutants that lack enzyme I or HPr show depressed motility, and the *crr* mutations that map adjacent to the *pts* operon also influence bacterial motility. It is believed that some of these effects are not mediated by cyclic AMP. The observation that the PTS regulates motile behavior can be rationalized physiologically; if a bacterium finds itself in a medium rich in sugar substrates of the PTS, it does not require motility to propel it to a more favorable environment. In fact, expenditure of energy for motility under these circumstances might be wasteful and therefore to the detriment of the organism. The observation that the PTS controls bacterial motility suggests that a primary function of the flagellum is in carbon and energy acquisition.

The second regulatory function of the PTS has been referred to above. The PTS controls the activities of several non-PTS sugar permeases (30–33). Of equal interest is the observation that the PTS regulates the activity of the cyclic AMP-synthetic enzyme, adenylate cyclase. Thus, addition of a sugar substrate of the PTS to a bacterial cell suspension simultaneously inhibits the activities of adenylate cyclase and a number of bacterial permease systems (7, 28, 31). This observation also can be rationalized teleonomically. It is well known that expression of the genes that code for catabolic enzyme systems, such as those responsible for lactose metabolism in *E. coli*, are subject to dual control by two small cytoplasmic molecules, inducer and cyclic AMP. Cellular inducer levels are determined by the activities of the specific permeases responsible for inducer uptake, and the intracellular concentration of cyclic AMP is determined, in part, by the activity of adenylate cyclase. Coordinate control of inducer uptake and cyclic AMP synthetic rates therefore provides a dual mechanism for regulating the rates at which non-PTS sugar catabolic enzyme systems are synthesized, and hence, a powerful mechanism for controlling rates at which these carbohydrates are metabolized. Worthy of note is the fact that cyclic AMP functions in this capacity as a second messenger or indicator of the availability of carbon and energy.

An example of the coordinate regulation of permease function and adenylate cyclase activity is shown in Fig. 3 (25). Figure 3A shows relative rates of glycerol uptake, whereas Fig. 3B illustrates cyclic AMP production plotted as a function of the concentration of methyl α-glucoside, a non-metabolizable PTS sugar, in

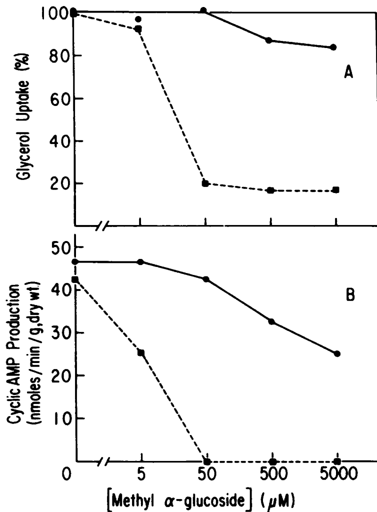


FIG. 3. *Illustration of the coordinate regulation of the glycerol permease (A) and adenylate cyclase (B) in intact cells of S. typhimurium which exhibit wild-type properties with respect to the proteins of the PTS. Solid lines show data for cells grown in galactose minimal medium; dashed lines illustrate results obtained with glucose-grown cells. Results are plotted as a function of methyl α-glucoside concentration. The figure was reproduced from reference 26 with permission.*

the extracellular medium. Data are included for a mutant of *S. typhimurium* that lacks the enzyme, cyclic AMP phosphodiesterase. The cells were grown under two different sets of conditions. The solid lines show data for cells grown in minimal galactose medium, conditions that do not result in induction of the synthesis of the enzyme II^{glc}, whereas the dashed lines show data for the same bacterial strain grown in glucose minimal medium. The latter cells possess elevated levels of enzyme II^{glc} activity (26). It can be seen that when cells possessed basal activity of the glucose enzyme II, the glycerol permease and adenylate cyclase were subject to only weak inhibition, even by high concentrations of methyl α-glucoside. In contrast, the same sugar was strongly inhibitory if the enzyme II^{glc} was present in increased amounts. In fact, a concentration of 50 μM methyl α-glucoside nearly completely inhibited both adenylate cyclase and glycerol uptake under these conditions. Figure 3 therefore illustrates the coordinate regulation observed when the amount of one of the proteins of the PTS, the enzyme II^{glc}, is varied.

As a result of recent genetic, biochemical, and physiological studies, a specific, but highly speculative, mechanism has been proposed to explain PTS-mediated regulation of permease function and adenylate cyclase activity (22, 25, 35). This model is illustrated in Fig. 4. The left-hand portion of the figure is a reiteration of the phosphate transfer chain of the PTS. The phosphoryl group of PEP can be sequentially transferred to enzyme I, then to HPr, and finally to sugar in the presence of the components of the sugar-specific enzyme II complex. A key feature of this model is the postulate that there exists in the cell a central regulatory protein, termed RPr, which can be phosphorylated as a result of transfer of the phosphoryl moiety from phospho-HPr. Consequently, this protein is assumed to exist in the cell in two alternative states: as an underivatized protein and in a phosphorylated form. It is further assumed that adenylate cyclase and other carbohydrate permeases that are sensitive to regulation possess allosteric regulatory sites, which render their catalytic activities sensitive to regulation. The allosteric effector molecules are assumed to be the free and derivatized forms of RPr. Thus, the carbohydrate permeases may normally exist in an active configuration, but binding of free RPr to the allosteric site on the cytoplasmic surface of the permease would alter the conformation of the protein such that it would function with reduced efficiency. Transport activity would therefore be inhibited. In contrast, adenylate cyclase may normally exist in a relatively inactive state, which exhibits a low rate of cyclic AMP synthesis. Possibly this enzyme can be activated when the phosphorylated form of RPr binds to its allosteric regulatory site. Thus, according to the model illustrated in Fig. 4, the non-PTS sugar permeases are subject to negative control by free RPr, whereas adenylate cyclase is subject to positive control by phospho-RPr.

Let us consider the physiological consequences of this model. Suppose the bacterial cell is energy proficient (possesses a sufficient cellular pool of PEP) and is suspended in a medium that lacks a sugar substrate of the PTS. Under these conditions the energy-coupling proteins of the PTS, enzyme I and HPr, as well as RPr, will exist in their phosphorylated states. Consequently, little free RPr will be present in the cell, the non-PTS permeases should function at maximal capacity, and adenylate cyclase should exist as the activated complex. Inducer uptake and cyclic AMP synthesis will therefore occur at maximal rates. Let us now consider the consequences of the addition of a PTS sugar to the culture medium. The presence of such a sugar should initiate vectorial phosphorylation of the sugar where the immediate source of the phosphoryl group would be expected to be a phospho-enzyme III or phospho-HPr. But since enzyme I~P, HPr~P, and RPr~P are all postulated to be of high energy and in equilibrium

FIG. 4. *Proposed mechanism for the regulation of non-PTS carbohydrate permeases and adenylate cyclase by the PTS in E. coli and S. typhimurium. Abbreviations as in Fig. 1. RPr is the hypothetical regulatory protein, possibly the same as enzyme* III^{glc} *(22). See text for explanation.*

with one another, all of these proteins would be drained of their phosphoryl moieties provided that the activity of the sugar-specific enzyme II complex were sufficiently great. Consequently, phospho-RPr would be converted to free RPr. The regulatory consequences of this dephosphorylation process would be to deactivate adenylate cyclase and inhibit the carbohydrate permeases. The proposed mechanism provides an explanation for several observations (7, 25, 26, 28, 31), including those illustrated in Fig. 3.

Although some aspects of this model must be considered tentative, others appear to be well established. For example, work with temperature-sensitive mutants of *E. coli* has established that enzyme I functions as a catalytic component of the regulatory system, and this observation has led to the conclusion that a phosphorylation mechanism is involved (7). For example, heat inactivation of enzyme I renders adenylate cyclase and several non-PTS carbohydrate permeases hypersensitive to regulation by low concentrations of PTS sugars. These results established that the energy-coupling proteins of the PTS participate in both the primary and the secondary functions of the PTS: their activities determine both the rates of PTS sugar uptake and the extent to which regulation of non-PTS permeases and adenylate cyclase occurs. The regulatory functions of the PTS appear to be completely dependent on its group translocation function, although no reciprocal dependency of sugar translocation on regulation has been noted.

The experimental and theoretical considerations noted above provide insight into the possible evolutionary basis for the complexity of the PTS. With a less complex system, sugar transport and phosphorylation could occur, but the regulatory interactions described above could not. Consequently, the complexity of the PTS may have evolved, in part, in order to allow greater control over cellular physiology. We shall return to this point after a consideration of comparative aspects of PTSs found in divergent bacterial families.

DISTRIBUTION OF THE PTS IN THE PROCARYOTIC WORLD

The presence of a PEP-dependent sugar PTS in eucaryotic organisms has not been convincingly demonstrated. However, the system has been found to be widely distributed in the procaryotic world (24). Table 2 summarizes the information presently available. The left-hand column lists bacterial genera in which a PTS has been demonstrated, whereas the right-hand column indicates bacterial genera in which PEP:sugar phosphotransferase activity has been sought but not found. Included among the organisms that possess a general PTS, capable of phosphorylating several sugars, are species of widely divergent bacterial groupings. The enteric bacteria such as *E. coli* and *S. typhimurium*

are found in this group (22), as are marine bacteria such as *Beneckea harveyi* and *Photobacterium fischeri* (13). Both gram-negative and gram-positive organisms, divergent species of *Mycoplasma* (8), and the freshwater prosthecate bacterium *Ancalomicrobium adetum* (34) are also listed. The PTS in this last organism is of particular interest since the entire enzyme complex appears to be membrane associated (34).

Among bacteria apparently lacking PEP-dependent sugar phosphorylation activity, one finds an equally heterogeneous group of gram-positive and gram-negative organisms (Table 2). A careful comparison of the two lists reveals that whereas most of the organisms on the right are strict aerobes, those on the left are obligate or facultative anaerobes, with only a few exceptions (i.e., *Bacillus subtilis* [12], *Arthrobacter pyridinolis* [20], and several *Pseudomonas* species [4, 36]). In fact, a generalization appears valid: those organisms that appear capable of metabolizing sugars via anaerobic glycolysis usually possess the ability to phosphorylate sugars by a PEP-dependent mechanism, whereas those that normally metabolize sugars aerobically via the Entner-Doudoroff pathway usually accumulate sugars by active transport mechanisms. This observation can be readily rationalized. A principal end product of anaerobic glycolysis is PEP; 2 mol of PEP is generated per mol of sugar metabolized via this pathway. Consequently, if the sugar is metabolized via glycolysis, energy for the uptake of additional sugar molecules by the PTS will always be available. In contrast, the principal energy sources generated by alternative sugar metabolic pathways are ATP and a proton electrochemical gradient (35, 42). Since these two forms of energy are available to the aerobically grown cell and serve to energize active transport systems, it is teleologically reasonable that many strict aerobes should accumulate carbohydrates by active transport mechanisms.

TABLE 2. *Distribution of the PTS in the procaryotic kingdom*

General PTS present		PTS absent	
Group	Genera	Group	Genera
8	*Beneckea*	1	*Rhodomicrobium*
	Escherichia	4	*Caulobacter*
	Klebsiella		*Hyphomicrobium*
	Photobacterium		*Prosthecomicrobium*
	Salmonella	7	*Azotobacter*
	Serratia	12	*Thiobacillus*
9	*Bacteroides*	14	*Micrococcus*
14	*Staphylococcus*	17	*Mycobacterium*
	Streptococcus		*Nocardia*
15	*Bacillus*		
	Clostridium		
16	*Lactobacillus*		
17	*Arthrobacter*		
19	*Mycoplasma*		
4	*Ancalomicrobium*		
Fructose-specific PTS present			
1	*Rhodopseudomonas*		
	Rhodospirillum		
	Thiocapsa		
	Thiocystis		
7	*Pseudomonas*		
	Alcaligenes		
Mannitol-specific PTS present			
5	*Spirochaeta*		

Any particular facultatively anaerobic bacterial species will, in general, phosphorylate some carbohydrates by the PTS-mediated group translocation process, whereas other sugars will be phosphorylated by ATP-dependent, kinase-catalyzed reactions (Table 1). Moreover, a sugar transported by one mechanism in one organism may be transported by a quite different mechanism in another organism. The mechanisms used are probably determined by several distinct considerations. First, it has been suggested that the relative affinities and specificities of the transport systems for their substrates, together with the availability of the same sugars, may influence the evolutionary pattern (see reference 2). Second, the pathways by which the carbohydrate is metabolized and the end products of metabolism (which become available for further uptake of sugar) may determine the preferred uptake mechanism, as discussed above. Third, the ease with which metabolism of the sugar is initiated may play an additional determinative role. In regard to this last possibility, if a sugar such as glucose is metabolized directly via glycolysis and does not require induced catabolic enzyme synthesis, then it should be an exceptionally favorable carbon source for growth. It can be utilized with minimal energy expenditure. On the other hand, sugars such as maltose, melibiose, and lactose must be hydrolyzed to their constituent monosaccharides before metabolic interconversion can be initiated. Consequently, utilization of one of these carbon sources requires the induced synthesis of the carbohydrate-specific catabolic enzymes. Since the utilization of these carbohydrates requires greater energy input, they should be less desirable sources of carbon. Furthermore, since the PTS regulates the utilization of non-PTS carbohydrates, the organism would benefit if the simpler sugars were substrates of the PTS whereas less readily utilized sugars were not. This suggestion is in agreement with the observation that numerous facultative anaerobes utilize simple hexoses via the PTS whereas disaccharides are accumulated by alternative transport mechanisms (Table 1).

In addition to the "general" PTSs characterized in many bacterial genera, a few evolutionarily divergent organisms have been found to possess sugar-specific systems with structural features and genetic regulatory mechanisms which differ markedly from those of enteric bacteria. For example, several species of the *Athiorhodaceae* (the nonsulfur purple photosynthetic bacteria) possess a fructose-specific PTS (27), whereas certain free-living spirochetes apparently possess a mannitol-specific PTS (29, 29a). In the sections that follow, I discuss the structural and genetic regulatory features of PTSs found in three organisms: *S. typhimurium* (or *E. coli*), *Rhodopseudomonas sphaeroides*, and *Spirochaeta aurantia*. Partly on the basis of the information considered, I shall postulate a pathway by which the complex PTS may have evolved.

REGULATION OF GENES CODING FOR THE PROTEIN CONSTITUENTS OF THE PTS IN *S. TYPHIMURIUM* AND *E. COLI*

The enzymatic pathways responsible for the initiation of glucose, fructose, and mannitol catabolism in *S. typhimurium* and *E. coli* are schematized in Fig. 5. All three sugars are transported and phosphorylated at the expense of cytoplasmic PEP via the PTS. The sugar phosphates that result are further converted to fructose 1,6-diphosphate, which can be metabolized via the Embden-Meyerhof glycolytic pathway.

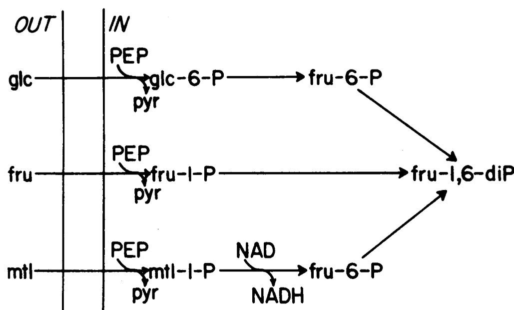


FIG. 5. *Schematic depiction of the initial steps in the catabolism of glucose (glc), fructose (fru), and mannitol (mtl) in S. typhimurium.*

The relevant proteins of the PTS which participate in the group translocation process are indicated in Fig. 6. As noted earlier, enzyme I and HPr are the general energy-coupling proteins of the system, required for the PEP-dependent phosphorylation of all sugars. The sugar-specific proteins of the system, the enzymes II and III, are also indicated in the branching phosphate transfer chain depicted in Fig. 6. The presence of an enzyme III^{mtl} is still hypothetical in enteric bacteria, although such a protein has been demonstrated in *Staphylococcus aureus* (38).

To gain insight into the mechanisms by which expression of the genes that code for the protein constituents of the PTS are regulated, enzyme induction studies have been performed (26). Table 3 summarizes relative extents of induction for several enzymes after growth of the cells in the presence of glucose, fructose, or mannitol. Levels of enzyme I and HPr were enhanced about threefold after growth on any one of these PTS sugars. In fact, any sugar substrate of the PTS exerted an inductive effect, although carbohydrates transported by alternative mechanisms did not. Genetic loss of a specific enzyme II rendered the sugar for which that enzyme II exhibited specificity noninducing. In all cases the induced syntheses of these two proteins were observed to be coordinate (10, 22, 32).

This behavior is to be contrasted with that for the sugar-specific components of the PTS. Synthesis of the glucose enzyme II was induced by glucose and, to a lesser extent, by mannitol, but not by fructose, whereas the fructose enzyme II and fructose-1-P kinase were specifically induced by growth in the presence of fructose. The mannitol enzyme II and mannitol-1-P dehydrogenase were induced about 20-fold when mannitol was included in the growth medium (26).

The results show that expression of the genes coding for the sugar-specific components of the PTS are regulated independently of the genes coding for enzyme I and HPr and that the sugar-specific catabolic enzymes are induced co-

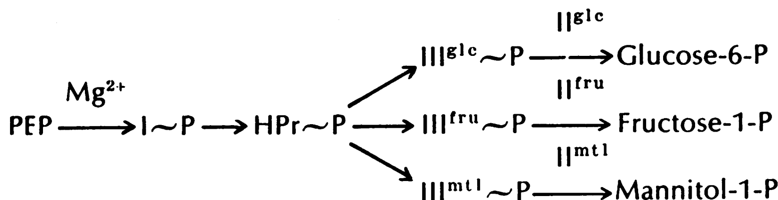


FIG. 6. *Diagrammatic representation of the branched phosphate transfer chain of the PTS in S. typhimurium. The figure is meant to illustrate the functions of the general, non-sugar-specific proteins of the PTS, enzyme I and HPr, as well as the sugar-specific proteins, enzymes II and III. The existence of an enzyme III^{mtl} has not been demonstrated in S. typhimurium and is therefore hypothetical.*

ordinately with the corresponding enzymes II. It therefore appears that each of the sugar-specific proteins of the PTS is coded for by a gene that is included within a regulon coding for the corresponding catabolic enzyme system.

This conclusion has been substantiated by extensive genetic and physiological studies. To illustrate this fact, the locations of relevant genes on the genetic linkage map of *S. typhimurium* are indicated in Fig. 7. Near the bottom of the circular map, at about 80 min, one finds an operon that encompasses the *ptsH* gene, which codes for HPr, and the *ptsI* gene, which codes for enzyme I. All available evidence indicates that these two genes comprise part of an operon (10, 22, 32). Interestingly, adjacent to the *pts* operon is a set of regulatory genes that have been designated *crr* for carbohydrate regulation resistance. Three such genes, *crrA*, *crrB*, and *crrC*, have been tentatively identified. Each of these genes appears to perform a specific function in the cell: the *crrA* gene regulates the utilization of several non-PTS carbohydrates (30) and has been postulated to code for a central regulatory protein termed RPr (see Fig. 4); the *crrB* gene apparently controls the utilization of certain Krebs cycle intermediates and amino acids, which feed into the Krebs cycle (R. C. Tuttle and M. H. Saier, Jr., Fed. Proc. **35**:1586, 1976); and the *crrC* gene influences cellular mo-

TABLE 3. *Induction of sugar catabolic enzymes in E. coli*

Enzyme	Induction after growth on:		
	Glucose	Fructose	Mannitol
Enzyme I	+ (3×)	+ (3×)	+ (3×)
HPr	+ (3×)	+ (3×)	+ (3×)
Enzyme II^{glc}	+ (5×)	−	± (3×)
Enzyme II^{fru}	−	+ (10×)	−
Fructose-1-P kinase	−	+ (10×)	−
Enzyme II^{mtl}	−	−	+ (20×)
Mannitol-1-P dehydrogenase	−	−	+ (20×)

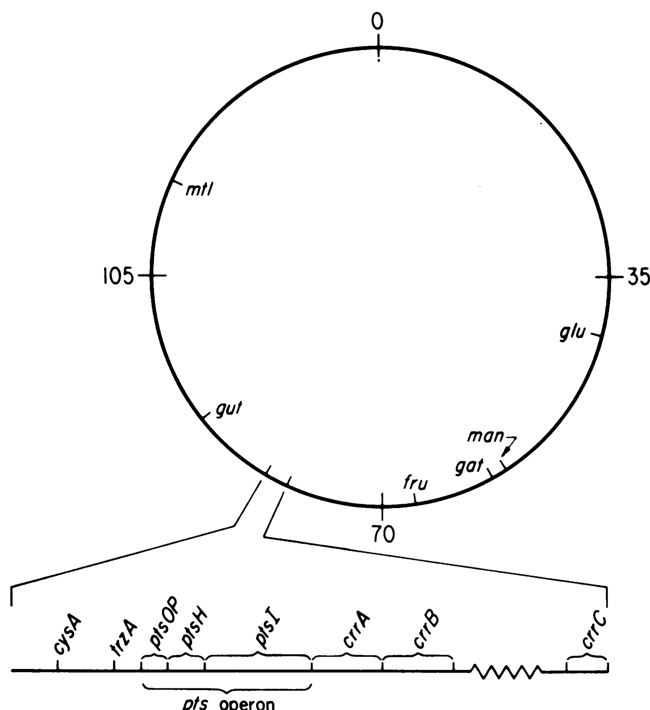


FIG. 7. *Genetic linkage map of S. typhimurium. The figure shows the approximate positions of the known genes of the PTS on the Salmonella chromosome. Genetic designations are as follows: ptsOP, ptsH, and ptsI, operator-promotor region, HPr structural gene, and enzyme I structural gene of the pts operon, respectively; crrA, crrB, and crrC, three regulatory genes of the PTS; mtl, gut, gat, and fru, operons including the genes that code for the enzymes which catalyze the initial catabolic steps for mannitol, glucitol, galactitol, and fructose degradation, respectively. The genes for the respective enzymes II are included in these operons. man and glu are the structural genes for enzyme* II^{man} *and enzyme* II^{glc}*, respectively. Genetic nomenclature is in accordance with recommendations of Lin (21). The genes coding for some of the enzymes II have been mapped only in E. coli (3, 10) and are assumed to be in the same locations on the Salmonella linkage map.*

tility (Tuttle and Saier, unpublished data). All of these genes appear to map to one side of the *pts* operon on the *Salmonella* linkage map.

Genes that code for the sugar-specific components of the PTS are found at different locations on the bacterial chromosome (Fig. 7). In general, these genes map together with those that code for the corresponding sugar-specific catabolic enzymes, but they map distant from the operons and genes that code for enzymes catalyzing the initial steps in the catabolism of other sugars. These observations are consistent with the results of Table 3 showing that each of the sugar-specific enzymes II is coded for by a gene subject to independent induction by the sugar substrate for which that enzyme II exhibits specificity.

PROPERTIES OF A FRUCTOSE-SPECIFIC PTS IN PHOTOSYNTHETIC BACTERIA

The nonsulfur purple bacterium *R. sphaeroides* can utilize glucose, fructose, and mannitol as primary sources of carbon for growth. Of these carbon sources, glucose and mannitol appear to cross the plasma membrane without modification (Fig. 8). Cytoplasmic glucose is phosphorylated with ATP and a constitutively synthesized glucokinase, whereas mannitol is first oxidized to fructose by nicotinamide adenine dinucleotide and mannitol dehydrogenase, and the resultant hexose is phosphorylated to fructose 6-P by a constitutive fructokinase (unpublished data). Because this organism lacks the enzyme, phosphofructokinase (9), these two sugars must be metabolized via the Entner-Doudoroff pathway. Of the sugars tested, only fructose is phosphorylated by a PEP-dependent mechanism (27), and only this sugar can be metabolized via glycolysis.

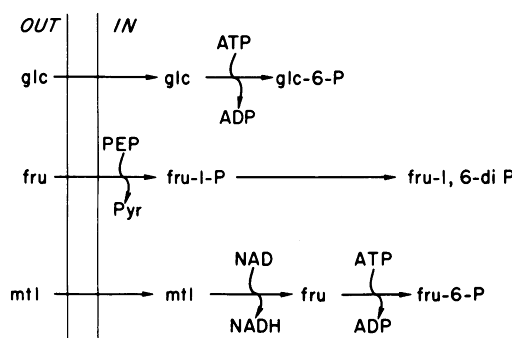


FIG. 8. *Schematic representation of the initial pathways for the metabolism of glucose (glc), fructose (fru), and mannitol (mtl) in R. sphaeroides. Because this organism lacks phosphofructokinase, glucose and mannitol, which give rise to hexose-6-phosphates, are metabolized via the Entner-Doudoroff pathway. Only fructose is converted to fructose 1,6-diphosphate and metabolized via the Embden-Meyerhof pathway.*

Preliminary structural analyses of the PTS in *R. sphaeroides* revealed the presence of only two catalytic proteins (Fig. 9). Both enzymes were required for transfer of the phosphoryl moiety of PEP to the 1-hydroxyl group of fructose. One of these proteins was an integral membrane constituent, termed R-P, and the other, designated R-S, was a peripheral membrane protein which could be released easily from the membrane in a totally water-soluble form (27). By analogy with the PTSs in other bacteria, the system was presumed to catalyze the transmembrane transport as well as the phosphorylation of fructose.

To gain information about the regulation of the expression of the genes that code for these proteins, induction studies were performed (Table 4). The two proteins of the *Rhodopseudomonas* PTS, together with fructose-1-P kinase, were induced about 10-fold by growth in the presence of fructose. Other sugars were without effect. Comparable studies with a strain of *Rhodospirillum rubrum* revealed that in this closely related species, synthesis of these three proteins was likewise induced, but only about threefold. It appeared that in both organisms the expression of the genes coding for these proteins was subject to coordinate regulation and that they therefore might comprise a single operon or regulon. For comparative purposes, Table 4 includes induction data for the glucose permease and the mannitol catabolic enzyme systems, neither of which was regulated coordinately with the fructose-specific proteins.

PROPERTIES OF A MANNITOL-SPECIFIC PTS IN *S. AURANTIA*

Figure 10 indicates the pathways for the initiation of sugar metabolism in *S. aurantia*. In this nonpathogenic, freshwater facultative anaerobe, both glucose and fructose appear to cross the plasma membrane without modification and are probably phosphorylated in the cytoplasm by ATP-dependent mechanisms. In *S. aurantia*, only mannitol appears to enter the cell by a PEP-dependent group translocation process. The product of the phosphorylation reaction, mannitol-1-P, is subsequently oxidized in the presence of mannitol-1-P dehydrogenase and nicotinamide adenine dinucleotide (29a).

Analyses of the mannitol-specific PTS in this organism revealed that it exhibited structural features in common with the PTS in enteric bacteria (Fig. 11). Thus, two soluble proteins were identified which were required for the PEP-dependent phosphorylation reaction. One of

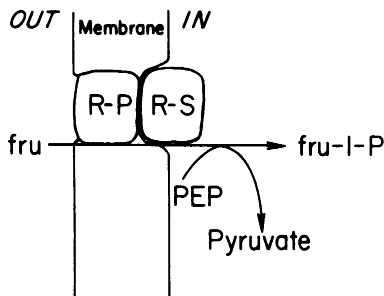


FIG. 9. *Diagrammatic representation of the structural constituents of the fructose-specific PTS in R. sphaeroides. R-P and R-S are the integral and peripheral membrane protein constituents of the system (27).*

TABLE 4. *Induction of sugar catabolic enzymes in R. sphaeroides*

Enzyme	Induction after growth on:		
	Glucose	Fructose	Mannitol
Glucose permease	+ (>10×)	–	–
Enzyme R-S	–	+ (10×)	–
Enzyme R-P	–	+ (10×)	–
Fructose-1-P kinase	–	+ (10×)	–
Mannitol permease	–	–	+ (>10×)
Mannitol dehydrogenase	–	–	+ (>10×)

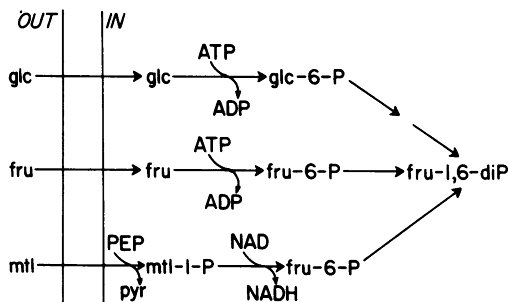


FIG. 10. *Representation of the initial pathways for the metabolism of glucose (glc), fructose (fru), and mannitol (mtl) in S. aurantia. Only mannitol is apparently utilized via a PEP-dependent mechanism in this organism (22).*

these was similar in size and physicochemical properties to enzyme I from *S. typhimurium*. The other protein was heat stable and of low molecular weight, being only slightly larger than HPr from enteric bacteria. Moreover, purified HPr from *S. typhimurium* could substitute for the spirochete protein if included in the assay mixture at a 100-fold-higher concentration than required to saturate in the homologous system (29a). These results clearly indicated that the low-molecular-weight protein from *S. aurantia* was the functional analog of HPr from *Salmonella*. The third enzymatic component of the spirochete PTS was an integral membrane constituent, shown to function as the sugar recognition component of the system (29). This protein was therefore analogous to enzyme II from enteric bacteria.

Although these results clearly showed that the components of the mannitol-specific PTS in *S. aurantia* exhibited some structural similarities with the *Salmonella* system, induction studies revealed that the genetic regulatory pattern was distinctly different (Table 5). When cells were grown with glucose or maltose as the carbon source, very low basal activities were observed for the three protein constituents of the PTS and mannitol-1-P dehydrogenase. Growth in fructose medium markedly enhanced all four activities, and maximal induction, amounting to a 100-fold increase over basal activity, was observed when the cells were grown in the presence of mannitol (29a). Table 5 also includes comparative data showing that whereas the glucose permease was synthesized constitutively in this organism, the fructose permease was specifically

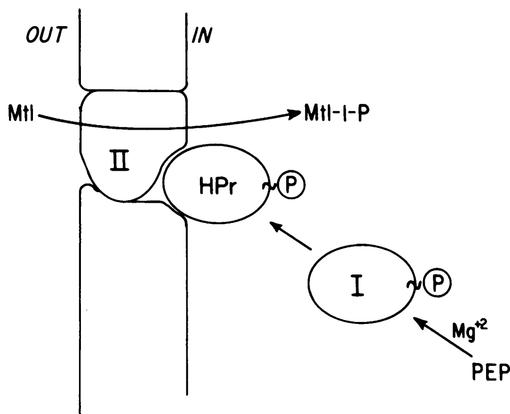


FIG. 11. *Schematic representation of the structural constituents of the mannitol-specific PTS in S. aurantia. I, HPr, and II represent enzyme I, HPr, and enzyme II^{mtl}, the three known constituents of the spirochete PTS.*

TABLE 5. *Induction of sugar catabolic enzymes in S. aurantia*

Enzyme	Induction after growth on:		
	Glucose	Fructose	Mannitol
Enzyme I	–	± (50×)	+ (100×)
HPr	–	± (50×)	+ (100×)
Enzyme II^{mtl}	–	± (50×)	+ (100×)
Mannitol-1-P dehydrogenase	–	± (50×)	+ (100×)
Glucose permease	+ (constitutive)	+	+
Fructose permease	–	+ (>10×)	–

induced when the cells were grown in the presence of fructose.

The coordinate induction pattern observed for the three proteins of the *Spirochaeta* PTS and mannitol-1-P dehydrogenase suggest that all four proteins are coded for by genes comprising a single operon or regulon. The data are in accord with the suggestion that these proteins function exclusively in mannitol metabolism.

PROPOSED PATHWAY FOR THE EVOLUTION OF THE EUBACTERIAL PTS

Comparative molecular biology allows us to view the end products of divergent pathways of evolution and to gain insight into the nature of the primordial genetic apparatus which gave rise to complex enzymatic and regulatory systems. This approach appears particularly useful in view of the fact that rates of evolutionary divergence can differ greatly depending on the degree of environmental stress experienced by an organism. Consequently, slowly evolving "splinter" groups of organisms may retain a biological system of the primordial ancestor, whereas groups comprising the mainstream of evolutionary advance may modify the system extensively. Based on the available information, most of which is discussed in this review, I propose that the complex eubacterial PTS evolved from a much simpler genetic system, as outlined in Fig. 12. It should be emphasized at the onset that this hypothetical scheme is based on limited evidence and that alternative possibilities exist.

The early ancestor of the PTS may have been a small, simple, inefficient, soluble sugar-phosphorylating polypeptide which increased in size and catalytic potency through evolutionary history. As a result of gradual nucleotide substitution in the structural gene and consequent substitution of hydrophobic amino acid residues for hydrophilic amino acid residues in the protein, this polypeptide chain became associated with the hydrophobic matrix of the cell membrane. By introducing appropriate amino acid substitutions, the polypeptide chain must have become oriented in a highly specific fashion, such that the sugar substrate bound to the external surface of the membrane protein while the product of catalysis, the sugar phosphate, was released into the cytoplasm. Since early procaryotic organisms found themselves in a strictly anaerobic environment, and aerobic metabolism became possible only late in the evolutionary scheme, it is not unreasonable to suppose that the first biological sugar transport systems were group translocation systems, and that active and facilitated diffusion systems evolved from these as a consequence of the partial loss of catalytic function. The introduction of additional types

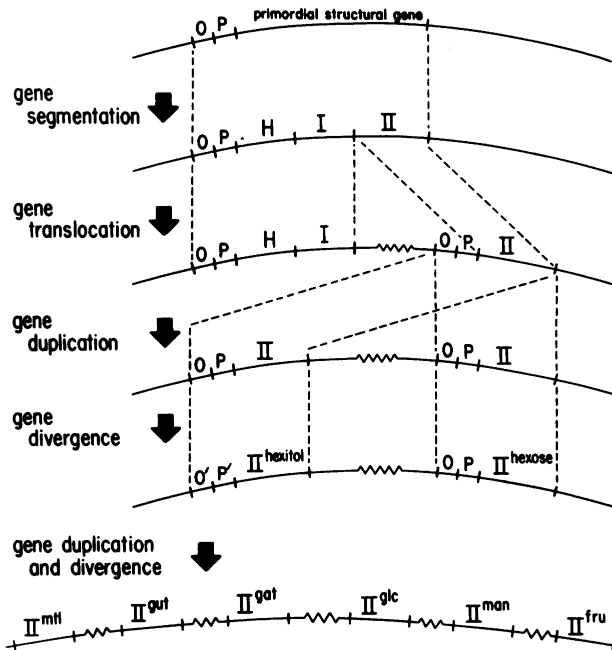


FIG. 12. *Hypothetical pathway for the evolution of the genetic elements coding for the protein constituents of a complex PTS such as that found in S. typhimurium. See text for explanation.*

of transport systems, which fed into alternative metabolic pathways, introduced the need for regulatory controls and, consequently, for increased degrees of complexity.

There is some precedence for assuming that a single enzyme might be capable of catalyzing group translocation. Present-day enteric bacteria possess a membrane-associated enzyme that appears to translocate adenine across the plasma membrane and derivatize it (14). This enzyme (adenine:phosphoribosyl pyrophosphate phosphoribosyl transferase) apparently binds adenine on the external surface of the membrane and phosphoribosyl pyrophosphate on the inner surface of the membrane and, after catalysis, releases its two products, AMP and inorganic pyrophosphate, in the cytoplasm. This process therefore conforms to the definition of group translocation.

Another enzyme which apparently catalyzes group translocation binds adenosine on the outer membrane surface and releases adenine into the external medium, but translocates the ribose moiety across the membrane (23). The intracellular product released by this enzyme is ribose-1-P where the phosphoryl moiety is derived from inorganic phosphate. These observations render plausible the conception of a primordial PTS that consisted of a single polypeptide chain and catalyzed transmembrane sugar transport and phosphorylation.

The first step in the sequence of events leading to a more complex PTS may have been gene segmentation (Fig. 12). Introduction of a chain-terminating codon in the primordial structural gene may have split the group-translocating polypeptide chain into two proteins. If the original protein was itself phosphorylated, using PEP as the phosphoryl donor, then the more hydrophobic integral membrane moiety of the segmented gene product must have retained the substrate binding site(s) while the more hydrophilic, peripheral moiety of the segmented gene product may have retained the phosphoryl acceptor site. The two protein subunits should both be essential for PEP-driven group translocation, and since both proteins share a common operator-promotor region, their syntheses should be subject to coordinate regulation. A second gene segmentation event may easily be postulated, thereby giving rise to a second soluble polypeptide chain. The resultant PTS would be coded for by three coordinately regulated structural genes, H, I, and II in Fig. 12. The postulated system may resemble the mannitol PTS in *S. aurantia*.

Exposure of the evolving bacterium to increasing numbers of extracellular nutritive and toxic carbohydrates may have introduced the desirability for an increased spectrum of transport capabilities without loss of stereospecific sugar recognition. This situation would require that the number of transport proteins be increased without broadening the sugar specificity of any one. In order for this goal to be reached, the evolving operon had to be split, and the gene coding for the integral membrane sugar recognition component had to be translocated to a new location on the bacterial chromosome. A new genetic regulatory region may have evolved adjacent to the translocated enzyme II gene, and consequently its expression was restored. This enzyme II gene may then have been duplicated so that evolutionary divergence could occur without loss of function (Fig. 12; 11).

Evolutionary divergence may have led eventually to two functional chromosomal structural genes coding for two distinct integral membrane proteins: one with a recognition site for hexoses of the pyranoside configuration and another that bound straight-chain polyols. Both retained the recognition sites for the soluble proteins of the PTS, and, consequently, both catalyzed group translocation. The resultant genetic material (Fig. 12) coded for the first complex PTS.

In order to complete the evolutionary scheme to give rise to a modern eubacterial PTS, the process of gene duplication and divergence must be repeated several times. Additional gene segmentation events may have created the structural genes for the sugar-specific enzymes III. Thus, the primordial hexitol enzyme II gene would give rise to genes that code for the enzymes II exhibiting specificity toward mannitol, glucitol, and galactitol, whereas the primordial hexose enzyme II gene would evolve into the genes coding for the enzymes II^{glc}, II^{man}, and II^{fru}.

Evidence for such an evolutionary pattern in the case of the hexitols has recently appeared (18, 19). Genetic and physiological characterization of the operons coding for the mannitol, glucitol, and galactitol operons in *E. coli* revealed that each of these genetic units consists of at least three genes: one coding for the hexitol-specific enzyme II; a second coding for the corresponding hexitol-P dehydrogenase, and a third coding for a regulatory protein that controls expression of the operon. The available evidence indicated that the order of structural genes is the same in all three operons (18). This observation clearly suggests that all three operons were derived from a common ancestral genetic unit.

Preliminary evidence in favor of common ancestry for the mannose and glucose enzyme II complexes is also emerging. The two genes coding for these enzymes are induced in *S. typhi-*

murium to a similar extent, the patterns of induction are similar, and both enzymes bind glucose with high affinity (unpublished data). Further structural and functional analyses of the protein constituents of the PTS may provide additional information concerning the evolutionary origins of the components of this system.

CONCLUSIONS

The eubacterial PTS is an enzyme system whose organizational complexity reflects its functional diversity. In addition to a primary role in nutrient acquisition, the PTS serves a secondary role in the regulation of cellular carbon and energy metabolism. Available genetic and physiological evidence suggests that the complexity of the system arose in an orderly manner from a simpler primordial system in response to evolutionary pressure for greater control over physiological processes. Such a view suggests that coordinate evolution of the PTS and of metabolic variability gave rise to integrated, versatile, and highly adaptive microorganisms.

ACKNOWLEDGMENTS

Original research in my laboratory reported in this article was supported by National Science Foundation grant BMS73-06802 A01 and Public Health Service grant 1 R01 CA165521-01A1 MBY from the National Cancer Institute. I was supported by Public Health Service Research Career Development Award 1 K04 CA00138-02 from the National Cancer Institute.

Thanks are due to K. E. Nealson, S. E. Mills, I. P. Crawford, and J. A. Hoch for valuable suggestions and discussions.

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