

The Bacterial Phosphotransferase System: Structure, Function, Regulation and Evolution

In Memoriam to Dr. Jonathan Reizer

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The PTS: An Overview

Thirty-seven years ago, Kundig, Ghosh and Roseman reported the discovery of a novel sugar-phosphorylating system in *Escherichia coli* (Kundig *et al.*, 1964). The unique features of this phosphotransferase system (PTS) included the use of phosphoenolpyruvate (PEP) as the phosphoryl donor for sugar phosphorylation and the presence of three essential catalytic entities, termed Enzyme I, Enzyme II and HPr (heat-stable, histidine-phosphorylatable protein). The discovery of this system provided an explanation for pleiotropic carbohydrate-negative mutants of *E. coli* described as early as 1949 (Doudoroff *et al.*, 1949).

In 1964, the three recognized activities of the PTS were presumed to correspond merely to three proteins. We now recognize dozens of PTS proteins in *E. coli* as well as hundreds of PTS proteins in other bacteria. Numerous genes encoding these proteins have been fully sequenced, and their phylogenetic relationships have been defined.

In 1964, a single function for the PTS, namely sugar phosphorylation, was known. Thirty-seven years later we find that this system plays roles in many surprising aspects of bacterial cellular physiology. Established primary functions of the system include sugar reception, transport and phosphorylation, whereas secondary functions include a variety of ramifications of metabolic and transcriptional regulation (Saier *et al.*, 1989, 1994; Saier and Reizer, 1994; Stülke *et al.*, 1998; Stülke and Hillen, 1998). Targets of regulation include (i) carbohydrate catabolic enzymes, sugar permeases and the cyclic AMP biosynthetic enzyme, adenylate cyclase, regulated allosterically by the IIA^{Glc} PTS protein in enteric bacteria; (ii) glycogen phosphorylase, regulated by the HPr protein in *E. coli*, (iii) the Mlc transcription factor, regulated by the glucose-specific permease, IICB^{Glc} in enteric bacteria; (iv) a variety of non-PTS transport systems, a sugar-phosphate phosphatase which controls the process of inducer expulsion, and the PTS itself, regulated by HPr(ser-P) in low G+C Gram-positive bacteria; (v) transcriptional activators and antiterminators regulated by direct phosphorylation in both enteric and Gram-positive bacteria; and (vi) carbohydrate catabolic enzymes and permeases, also regulated by direct phosphorylation in Gram-positive bacteria.

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PTS auxiliary proteins such as the fructose repressor, FruR, and the Mlc transcription factor are believed to control transcription of the PTS genetic apparatus as well as of genes encoding central pathways of carbon metabolism in enteric bacteria (Plumbridge, 1999; Saier and Ramseier, 1996). These pathways include glycolysis, the Krebs cycle, electron transport, the glyoxylate shunt, gluconeogenesis, and possibly the Entner-Doudoroff pathway. Both *pts* and *fruR* mutants of *Salmonella typhimurium* are greatly attenuated for virulence in mice (Groisman and Saier, 1990; Saier and Chin, 1990).

Genetic evidence has indicated that other processes including the net production of carbon and energy storage sources such as poly- β -hydroxybutyrate (Pries *et al.*, 1991) and the control of σ -dependent transcription of nitrogen metabolic genes in numerous Gram-negative bacteria (Merrick and Coppard, 1989; Reizer *et al.*, 1992) are also controlled by the PTS. Moreover, the biochemical detection of novel, functionally uncharacterized PTS proteins in bacteria as diverse as *Ancaalmicrobium adatum* (Saier and Staley, 1977), *Spirochaeta aurantia* (Saier *et al.*, 1977), *Acholeplasma laidlawii* (Hoischen *et al.*, 1993), *Listeria monocytogenes* (Mitchell *et al.*, 1993) and several antibiotic-producing species of *Streptomyces* (Titgemeyer *et al.*, 1994) suggests the involvement of PTS proteins in cellular processes distinct from those currently recognized. It is worth noting that other families of transport systems such as the family of ATP-binding cassette (ABC)-type permeases (Higgins, 1992), and the major facilitator superfamily (Pao *et al.*, 1998) apparently do not participate in metabolic and transcriptional regulation, at least to the extent observed for the PTS.

In this PTS symposium, dedicated to the memory of Dr. Jonathan Reizer, we shall review some current research on the PTS, discuss the multifaceted structural and functional aspects of the system and attempt to provide a realistic forecast of future discoveries. The potential benefits of PTS research seem unlimited. Its study will undoubtedly advance our fundamental knowledge of molecular evolution, will contribute to our understanding of prokaryotic physiology and pathogenesis, will allow major advances in biotechnology, and will result in the development of agents capable of effectively combating harmful microorganisms.

Jonathan Reizer: A Scientific History

On December 31, 1999, Dr. Jonathan Reizer died of cancer at the age of 59. Most of his scientific career was devoted to studies of the PTS. Jonathan was a graduate of the Hebrew University in Jerusalem, receiving his Ph.D. in 1978 for studies dealing with properties of the cytoplasmic

membrane of a thermophilic *Bacillus* species. Already in these early years, molecular transport and the PTS were topics of interest to Jonathan. In his short postdoctoral studies at Thomas Jefferson University in Philadelphia, Jonathan continued his studies on neutral amino acid and sugar transport in Gram-positive bacteria. He discovered a novel regulatory mechanism termed "inducer expulsion" in which cytoplasmic sugar phosphates are hydrolyzed and the sugar moiety is expelled. This phenomenon was to be the focus of his studies for several years to come.

After further postdoctoral studies at Brown University in Rhode Island, Jonathan joined my research group at the University of California in San Diego as a postdoctoral fellow. In this environment he flourished, publishing several important papers in a three-year period (1982-1985) dealing primarily with transport and its regulation in both Gram-positive and Gram-negative bacteria. Following another productive three-year period (1985-1988) at the National Institutes of Health, Jonathan returned to UCSD where he remained for the rest of his life as a research biologist. In addition to his well-known biochemical, molecular genetic and physiological studies of the PTS, Jonathan purified many PTS proteins to homogeneity in preparation for collaborative 3-dimensional structural analyses by both x-ray crystallography and multidimensional NMR. Preparation of mutant forms of these proteins as well as ^{13}C and ^{15}N derivatives was a part of these efforts. Finally, in the last several years of his life, Jonathan and his wife Aiala mastered and applied bioinformatic tools to the identification and characterization of the PTS in many organisms. A major focus of his bioinformatic work dealt with the analysis of operons encoding PTS proteins in *Escherichia coli* (Reizer *et al.*, 1992, 1993, 1994, 1995, 1996a,b,c; see Reizer and Saier, 1997 for a review).

The JMMB PTS Symposium: Topics Included

As noted above, the PTS plays roles in many prokaryotic physiological processes, and several of these are the focus of this JMMB symposium. A description of the complete PTS in *E. coli* is the focus of the first article in this symposium, that by J. Tchieu *et al.* In the second article, by A. Peterkofsky and his coworkers, available 3-dimensional structural data for several PTS proteins, revealing their interactive interfaces, is evaluated. The next article, authored by Sir Hans Kornberg, deals with the regulation of fructose metabolism in *E. coli*, a longstanding interest of this investigator. Then follows an article by M. Esquinas-Rychen and B. Erni, in which the process of *E. coli* bacteriophage lambda infection, which depends on the protein constituents of the mannose Enzyme II complex of the PTS, particularly on the Enzyme IIC component, is analyzed. The article by J. Plumbridge and her coworkers reviews the recent exciting literature dealing with the direct involvement of the glucose Enzyme II complex in transcriptional regulation by the transcription factor, Mlc, which regulates several important genes encoding PTS and non-PTS proteins in enteric bacteria. The article by M. Kuroda and colleagues presents an insightful and up-to-date summary of the available evidence revealing that

in the galactoside (lactose and melibiose) permeases of *E. coli*, multiple sites of interaction with the regulatory PTS protein, IIA^{Glc}, account for the allosteric regulation of these transporters. Another very interesting regulatory function of the PTS concerns the control of glycogen metabolism. This topic, with a focus on regulation of the *E. coli* glycogen phosphorylase by HPr, is presented in the article by Y.-J. Seok and colleagues.

Remaining articles in this symposium deal with the PTS in Gram-positive bacteria. The first of these represent original research articles on the involvement of the PTS in the phenomenon of catabolite repression in the Gram-positive bacterium, *Bacillus subtilis*. The article by V. Monedero *et al.* provides evidence that the *cccA* gene, encoding cytochrome C₅₅₀, is subject to PTS-mediated catabolite repression, while the article by F. Penin and coworkers analyzes the propensity of the catabolite repression HPr-like protein, Crh, to undergo oligomerization. The article from the Titemeyer lab surveys the PTS proteins encoded within the *Corynebacterium diphtheriae* genome, revealing a surprising array of PTS constituents considering that no PTS homologues are encoded within the genomes of related *Mycobacterium* species. The review by Poolman and his collaborators summarizes the multifaceted ramifications of PTS regulation with emphasis on low G+C Gram-positive bacteria. Finally, the last symposium article by C.M. Kowolik and W. Hengstenberg, provide novel methodologies for studying PTS protein interactions in Gram-positive bacteria.

The papers included in this symposium summarize some of the fascinating topics of current research concerning the structures, functions and evolutionary relationships of proteins of the bacterial phosphotransferase system. The involvement of this complex system in sugar transport and a wide range of regulatory phenomena are summarized. We hope that this written symposium will stimulate interest in the multifaceted functions of the PTS in bacteria. It serves as a tribute to the accomplishments of Jonathan Reizer whose enthusiasm for the PTS was unexcelled.

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