The PTS System: As important for biofilm formation by *Vibrio cholerae* as it is for metabolism in *Escherichia coli*

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Abstract

The phosphoenolpyruvate transferase system (PTS) transports sugars and is poised to measure the metabolic status of the cell. This metabolic monitoring capacity has now been shown to have a central role in controlling biofilm formation by *Vibrio cholerae*, with at least three distinct mechanisms by which the PTS exerts this control.
Microbial biofilms are multicellular aggregates embedded in an exopolymeric matrix that contribute to survival in environmental niches such as rock surfaces in streams, plant roots, or the middle ear or teeth in the human body. Intense research effort has gone into identifying molecular mechanisms used by bacteria to build and regulate biofilms. In this issue of the *Journal of Bacteriology*, Houot et al. report on the identification of three different phosphoenolpyruvate phosphotransferase system (PTS) pathways that control biofilm formation by *Vibrio cholerae*. This study highlights how important the PTS is in controlling biofilm formation. While other studies with different bacterial species have shown that a mutation in a gene for a component of the PTS affects biofilm formation (1, 19, 22), these studies have not yet done a thorough analysis of which components of the PTS affect biofilm formation and whether phosphorylation of these proteins is required for regulation. The PTS can transport a variety of sugars, and this report highlights the importance of studying the role of the PTS in different growth media to reveal the full extent to which the PTS affects biofilm formation. Along with the recent work demonstrating how the PTS system can influence virulence gene expression in some bacterial species (14, 24, 27), the finding that the PTS has a profound effect on biofilm formation may be bringing out a renaissance in research on this sugar transport system that was first described in the 1960’s.

The PTS and *Vibrio cholerae*

The PTS is used for the transport of sugars and requires phosphate transfer from PEP to enzyme 1 (E1) to Hpr or Fpr and then to an EII complex (Fig. 1). A membrane-associated EIIB then transfers the phosphate from EIIA to the specific sugar that is transported across the membrane.
by the EIIC/D complex (7). The nature of the PTS is that, in the absence of transported sugars,
phosphate will accumulate on the protein components of the PTS. Thus, the phosphorylation
state of the PTS is an indirect read-out of the presence of PTS sugars and the metabolic status of
the cell. The PTS system has long been known to participate in regulation that impinges on
metabolism, including chemotaxis, inducer exclusion, and catabolite repression (7, 25).
Recently, it has also been implicated in regulating processes not directly related to metabolism
such as virulence gene expression.

In addition to this canonical PTS, many bacteria also possess a “nitrogen” PTS, which is
comprised of an EI component, an HPr component (called NPr), and an EIIA component (Fig.
1) (23). This “nitrogen” PTS is unique for the lack of an EIIB/C complex. This has lead to the
proposal that the “nitrogen” PTS would function solely in a regulatory capacity, but the precise
mechanism by which this would work is still unknown.

From bioinformatic analyses, *V. cholerae* would appear to possess both a canonical PTS and a
“nitrogen” PTS. For the canonical PTS, there is a single EI component, an HPr and FPr, and 8
different EIIA components. These EIIAs work with one of the many different EIIB/C complexes
in *V. cholerae* (11), each of which will transport a specific sugar, including glucose, fructose,
mannose, mannitol, and chitobiose among others (2, 11, 12). The *V. cholerae* “nitrogen” PTS
appears to be composed of an EI component, an NPr, and two EIIA<sup>Ntr</sup> components. Prior to the
work described in Hout et al., it was shown that the PTS system is required for intestinal
colonization and inhibits biofilm formation (11, 12). Specifically, the phosphorylation of Hpr or
FPr, which accumulates in the absence of a transportable PTS sugar, was required to inhibit expression of the biofilm matrix genes.

Vibrio cholerae biofilms

Control of biofilm formation by V. cholerae is complex. This complexity largely centers on the transcriptional control of the vps operon, which encodes the V. cholerae exopolysaccharide, also known as VPS. Expression of vps is critical for biofilm formation by V. cholerae (29). A multiple component, signal transduction cascade controls expression of vps, and includes the proteins VpsS, VpsR, VpsT and HapR (3, 6, 10, 16, 26, 28, 30). This protein cascade allows a range of signals to control biofilm formation either negatively or positively. For example, expression of vps is negatively regulated by quorum sensing, Ca\(^{2+}\), and cyclic AMP (4, 8, 10, 18, 30). Other molecules such as indole, bile acids and norspermidine stimulate vps expression (13, 15, 21). To this complexity, now enters three independent pathways for PTS to control vps expression. How all these signals are integrated to control biofilm formation by V. cholerae is unknown.

The three PTS pathways controlling biofilm formation

Consider that all PTS proteins required to transport glucose in Escherichia coli regulate a metabolic function. E1 interacts with CheA to control chemotaxis, HPr interacts with glycogen phosphorylase to control recycling of glycogen, EIIA\(^{Glc}\) interacts with non-PTS transporters to prevent transport of other sugars and the adenylate cyclase that makes cyclic AMP, and EIIB\(^{Glc}\) interacts with the transcription factor Mlc to control expression of the genes involved in uptake of glucose, just to list a few (7). The multifunctionality of the PTS system indicates that the
mechanism of PTS control of biofilm formation cannot readily be predicted from a finding that any single mutation in a gene for the PTS system affects biofilm formation. Furthermore, by studying which component of the PTS system is responsible for regulation, one can reveal, as the study of Houot et al. did, that PTS control of biofilm formation may be almost as complex as the PTS control of metabolism in *E. coli*.

The first pathway described for controlling *vps* expression in *V. cholerae* is active in cells grown on either a glucose minimal medium or a rich medium like LB. In this pathway, PEP-dependent, phosphorylated HPr or FPr inhibits *vps* expression (Fig. 1). PEP-dependent, phosphorylated HPr controls the activity of a group of antiterminator proteins and transcription factors in Gram-positive bacteria (9). The *vps* operon is not known to be controlled by transcription antitermination. Could the *V. cholerae* HPr/FPr be phosphorylating a component of the signal transduction cascade that controls *vps* expression? Phosphorylated PTS components accumulate in the absence of a transportable sugar, indicating that lack of a PTS sugar inhibits biofilm formation by *V. cholerae*.

The second pathway involves the EIIA\textsubscript{Glc} and EIIBC\textsubscript{Glc} components of the glucose-specific PTS and is active only in cells grown on LB medium (Fig. 1). EIIBC\textsubscript{Glc} inhibits *vps* transcription, and acts presumably as it does in *E. coli* (5), were unphosphorylated EIIBC\textsubscript{Glc} inhibits the activity of the transcriptional repressor Mlc. However, Mlc does not appear to control *vps* gene expression directly, but instead largely appears to work by potentiating EIIA\textsubscript{Glc} activity. How Mlc affects EIIA\textsubscript{Glc} activity is unknown, but the interesting question here is how does EIIA\textsubscript{Glc} stimulate *vps* expression. Unphosphorylated EIIA\textsubscript{Glc} of *E. coli* interacts with non-PTS transporters to inhibit
their activity (7). What new type of protein might future *V. cholerae* work reveal about the types of proteins that can interact with EIIA$^{Glc}$ proteins?

The third pathway diverges from components of the canonical PTS components to components of the far less understood PTS$^{Ntr}$. *V. cholerae* possess two EIIA$^{Ntr}$ homologs, and both contribute to inhibit *vps* expression (Fig. 1). It is interesting that whether these EIIA$^{Ntr}$ homologs are unphosphorylated or phosphorylated, they can still inhibit *vps* expression. Is there something upstream of EIIA$^{Ntr}$ homologs pathway that affects their ability to inhibit *vps* expression? Also, what is downstream of the EIIA$^{Ntr}$ homologs that allow these proteins to control *vps* transcription in *V. cholerae*? It will be interesting to know whether inhibiting *vps* expression occurs through interaction of EIIA$^{Ntr}$ with the K+ transporter as it does in *E. coli* (17).

Alternatively, EIIA$^{Ntr}$ could interact with one of the two-component regulatory systems that are known to control *vps* expression, similar to how EIIA$^{Ntr}$ binds and inhibits the activity of the KdpD histidine-protein kinase of *E. coli* (20).

The cumulated studies of the PTS tell us how well suited the PTS is to monitor the metabolic capability of the cells and control relevant pathways. It has been long known that the PTS could affect related metabolic pathways, chemotaxis, inducer exclusion, and catabolite repression. The finding that the PTS affects processes such as virulence and biofilm formation takes the centrality of the PTS to the physiology of the cell in a new direction and suggest new avenues for the development of antimicrobials.
References


Figure Legends

Figure 1. Model for how the PTS systems of *Vibrio cholerae* affects biofilm formation. Show in yellow is the sugar-dependent PTS and in blue is the nitrogen PTS. Green arrow and “T” lines indicated points at which a PTS component stimulates or inhibits, respectively, biofilm formation through controlling *vps* expression. The gray boxes indicate that either the unphosphorylated or phosphorylated protein can control *vps* expression.