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Review

The phospho*enol*pyruvate-dependent glucose–phosphotransferase system from *Escherichia coli* K-12 as the center of a network regulating carbohydrate flux in the cell

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ABSTRACT

The phospho*enol*pyruvate-(PEP)-dependent-carbohydrate:phosphotransferase systems (PTSs) of enteric bacteria constitute a complex transport and sensory system. Such a PTS usually consists of two cytoplasmic energy-coupling proteins, Enzyme I (EI) and HPr, and one of more than 20 different carbohydrate-specific membrane proteins named Enzyme II (EII), which catalyze the uptake and concomitant phosphorylation of numerous carbohydrates. The most prominent representative is the glucose–PTS, which uses a PTS-typical phosphorylation cascade to transport and phosphorylate glucose. All components of the glucose–PTS interact with a large number of non-PTS proteins to regulate the carbohydrate flux in the bacterial cell. Several aspects of the glucose–PTS have been intensively investigated in various research projects of many groups. In this article we will review our recent findings on a Glc-PTS-dependent metalloprotease, on the interaction of $EIICB^{Glc}$ with the regulatory peptide SgrT, on the structure of the membrane spanning C-domain of the glucose transporter and on the modeling approaches of *ptsG* regulation, respectively, and discuss them in context of general PTS research.

Introduction

Bacteria have the capacity to utilize a great variety of nutrients. Moreover, they can quickly adapt to continuously changing environmental conditions. In an environment of mixed available nutrients, bacteria generally have one preferred carbon source. This was first investigated in detail by Monod (1942), who described the phenomenon of diauxic growth, demonstrating that the enteric bacterium *Escherichia coli* primarily chooses glucose when exposed to a nutrient mixture of D-glucose and sorbitol. Since then, glucose has been regarded as the classical "preferred" carbon source and has been studied for decades in order to unravel the molecular mechanisms of carbon source-transport and its regulation. The major glucose transport system of *E. coli* was first described in 1966 when a glucose-specific phospho*enol*pyruvate (PEP)-dependent phosphotransferase system (PTS) was identified (Kundig et al., 1964). Since then, many different PTSs for the uptake of a great number of carbohydrates have been found in both Gram-negative and Gram-positive bacteria. Decades of research have established that the PTSs not only function as carbohydrate uptake systems, but that they also constitute highly sophisticated carbohydrate sensor systems, which control carbon and nitrogen metabolism, chemotaxis, biofilm formation and other bacterial responses to changing environmental conditions [reviewed in (Deutscher et al., 2004; Lengeler and Jahreis, 2009)].

Usually, a PTS consists of two cytoplasmic energy-coupling proteins, the Enzyme I (EI, gene *ptsI*) and the Histidine-containing protein (HPr, gene *ptsH*), and in particular for *E. coli* K-12 of a range of more than 20 different carbohydrate specific Enzymes II (EIIs), which catalyze concomitant transport and phosphorylation of the carbohydrate. The first step in the PTS-typical phosphorylation cascade is catalyzed by EI, a PEP-dependent protein-kinase. The use of PEP, an intermediate of the glycolysis, as a phosphoryl group donor couples carbohydrate transport and metabolism tightly. In the case of the glucose–PTS the phosphate group is subsequently transferred from EI∼P to HPr, from HPr∼P to the soluble $EIIA^{Glc}$ (sometimes also called $EIIA^{Crr}$, gene *crr*, part of the *ptsHIcrr* operon), and finally from $EIIA^{Glc}$∼P to the glucose-specific membrane protein $EIICB^{Glc}$ (gene *ptsG*), which mediates uptake and phosphorylation of glucose. All phosphotransfer reactions between PEP and the EIIB domains of any carbohydrate-specific EII are reversible. Only the final step, i.e., the transfer of the high-energy phosphate group to the substrate, is virtually irreversible. Thus, all PTSs of a single cell form a complex

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network, in which the phosphorylation levels of all components depend on the availability of the appropriate carbohydrates and the physiological state of the cell. As we will see in this review, cells use the information about the phosphorylation levels of the various PTS-proteins to control the cellular carbon flux.

When we started to investigate the regulation of the *ptsG* gene in 1999, it was believed, that the transcription of this gene occurs mainly constitutively, since it encodes the transport protein for one of the most important carbohydrates of the cell. Since then many studies have revealed that the regulation of *ptsG* expression is very complex [see Fig. 1; reviewed in (Böhm and Boos, 2004; Deutscher et al., 2004; Jahreis et al., 2008)]. Besides the specific glucose repressor Mlc [mnemonic for "makes large colonies"; gene *dgsA* (Hosono et al., 1995)], several different global transcription factors were identified. Among them are the cAMP-CAP complex, which activates *ptsG* transcription under carbohydrate starvation conditions (Jahreis, 2010), ArcA, a major transcription factor controlling the switch between aerobic and anaerobic growth in *E. coli* (Jeong et al., 2004), SoxS, which is responsible for *ptsG* induction under oxidative stress (Rungrassamee et al., 2008), two alternative sigma factors, $\boldsymbol{\sigma}^{32}$ for heat shock response (Shin et al., 2001) and $\boldsymbol{\sigma}^{S}$ for the expression of genes in the stationary growth phase (Seeto et al., 2004), and the small DNA-binding protein Fis (Shin et al., 2003), respectively. Moreover, we were able to identify an additional protein called MtfA [mnemonic for "Mlc titration factor", formerly called YeeI, gene *mtfA* (Becker et al., 2006)], which binds to Mlc and thereby indirectly affects *ptsG* transcription. In addition to these regulation mechanisms on the transcriptional level, *ptsG* expression has been found to be post-transcriptionally regulated by the modulation of its mRNA stability and by the regulation of the EIICB^{Glc} activity in response to the glycolytic flux in the cells by the so called SgrR, SgrS, SgrT system [mnemonic for "sugar transport related", genes *sgrR sgrS sgrT* (Morita et al., 2003)].

This review is divided into four parts: The first part deals with the major subject of our research project: The characterization of MtfA, a zinc-dependent metalloprotease. Its activity appears to be regulated in a glucose-dependent manner. The second part addresses the question concerning the relevance of a sophisticated control of *ptsG* expression. The third part is an overview regarding the post-transcriptional regulation of the glucose–PTS under intracellular carbohydrate-phosphate stress. The final part reviews the available data about the structure of EIICB^{Glc}.

Properties of the zinc-dependent-metalloprotease MtfA

The major specific regulator of *ptsG* expression is the repressor Mlc, which is inactivated by glucose in the medium. In contrast to other repressors, induction of Mlc is not catalyzed by direct binding of glucose or any other small molecular inducer. Instead, as part of a novel regulatory mechanism, the membrane-bound, non-phosphorylated EIICB^{Glc} binds Mlc. Thus, in the absence of glucose, Mlc binds to the operator/promoter region of *ptsG*, while in the presence of glucose, non-phosphorylated EIICB^{Glc} pulls the repressor away from its promoter, allowing induction and enhanced *ptsG* transcription (Lee et al., 2000; Nam et al., 2001; Tanaka et al., 2000; Zeppenfeld et al., 2000). In this manner the complex between the membrane-bound EIICB^{Glc} and the soluble DNA-binding protein Mlc, forms an efficient glucose sensing system. Perhaps not surprisingly, DNA-microarray experiments revealed a pleiotropic role of Mlc in the regulation of further carbohydrate transporters and catabolic enzymes including the *ptsHIcrr* operon itself in response to the presence of glucose [references in (Deutscher et al., 2004), and our unpublished results]. In order to identify new proteins involved in the regulation of *ptsG*, we established a genetic screen to detect mutants with reduced EIICB^{Glc} activity. For this purpose we used a "relaxed" *ptsG* mutant, which due to an S169F amino-acid substitution in the EIICB^{Glc} is capable of transporting mannose via the Glc-PTS, but only at a low rate. With this mutant we performed a Mini-Tn10Kan^{R} transposon mutagenesis. As mannose is a poor substrate for the EIICB^{Glc}, the phenotype on MacConkey mannose indicator plates was suitable to search for mutations, which decrease, but do not completely suppress the transport activity of the Glc-PTS just by looking for Glc^{+} Man^{-} cells. Using this screen we identified three different mannose-negative mutants, which still showed glucose fermentation. All of these mutants carried a different and unique transposon insertion in the open reading frame of unknown function *yeeI* (*mtfA*). A further analysis of these *yeeI* (*mtfA*) mutants revealed that they indeed exhibit a reduced *ptsG* expression and thus a reduced growth on a medium with glucose as a sole carbon source. On the other hand, overexpression of YeeI (MtfA) led to a constitutive *ptsG* expression. Moreover, using the surface plasmon resonance technique we demonstrated a tight direct protein–protein interaction between Mlc and YeeI (MtfA) with an apparent K_D of 14 ± 0.3 nM, providing an explanation for the observed constitutive *ptsG* expression by YeeI overexpression. Thus, we renamed the gene *yeeI* to *mtfA* (Becker et al., 2006). MtfA is widespread among very different bacterial species and forms a protein family of more than 350 family members (Pfam PF06167). MtfA-like proteins occur predominantly in proteobacteria (~88%), especially in their β- and γ-groups. But, they are also present in *bacteroidetes*, *cyanobacteria*, and *planctomycetes* (~11%). One remote eukaryotic homologue is present in *Nematostella vectensis* (Starlet sea anemone), a primitive animal (Qingping Xu et al., in preparation). Extensive structural studies on MtfA from *Klebsiella pneumoniae*, which shares 76% identical amino-acids with *E. coli* MtfA, revealed a strong overall similarity to zinc-dependent metalloproteases like the anthrax lethal factor. Using site directed mutagenesis we constructed MtfA mutants with substitutions of predicted catalytically important amino-acid residues, confirming the predicted protease activity of MtfA (Qingping Xu et al., unpublished). Interestingly, the glucose repressor Mlc is not a target for the protease activity of MtfA. On the contrary, in *in vitro* assays addition of Mlc to MtfA stimulates its protease activity. Thus, our current working model suggests, that in the absence of glucose, Mlc is released by the rephosphorylated EIICB^{Glc}, which has two consequences: First, Mlc represses *ptsG* gene expression (and the expression of other Mlc regulated genes) and secondly, free Mlc binds to MtfA, thereby enhancing its protease activity, leading to a readjustment of cellular metabolism to glucose-starvation conditions. Experiments are in progress to identify natural targets of MtfA.

Relevance for a sophisticated regulation of the *ptsG* transcription and EIICB^{Glc} activity

The key to understanding the role of the PTS in all its regulatory functions lies in the complex phosphorylation cycle of its protein kinase EI (Chauvin et al., 1996). Autophosphorylation of EI occurs only in its dimeric state. After phosphorylation, the dimers dissociate and subsequently transfer the phosphate groups to HPr. If the expression of the genes for all proteins of the PTS-phosphorylation cascade have been completely induced, this obligatory dimerization of EI monomers prior to autophosphorylation appears to be the rate limiting step in the EI phosphorylation cycle, since the rate of association/dissociation of EI is very slow in comparison to other protein kinases (Chauvin et al., 1996; Meadow et al., 2005). These results led to the following model of the PTS as a sensory system: All functional subunits of the PTS can exist in the phosphorylated or unphosphorylated form, depending on the available carbohydrate and the physiological state of the cell, which is for example