Regulatory Protein Phosphorylation in *Mycoplasma pneumoniae*

**A PP2C-TYPE PHOSPHATASE SERVES TO DEPHOSPHORYLATE HPr(Ser-P)**

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Among the few regulatory events in the minimal bacterium *Mycoplasma pneumoniae* is the phosphorylation of the HPr phosphocarrier protein of the phosphotransferase system. In the presence of glycerol, HPr is phosphorylated in an ATP-dependent manner by the HPr kinase/phosphorylase. The role of the latter enzyme was studied by constructing a *M. pneumoniae* hprK mutant defective in HPr kinase/phosphorylase. This mutant strain no longer exhibited HPr kinase activity but, surprisingly, still had phosphatase activity toward serine-phosphorylated HPr (HPr(Ser-P)). An inspection of the genome sequence revealed the presence of a gene (*prpC*) encoding a presumptive protein serine/threonine phosphatase of the PP2C family. The phosphatase PrpC was purified and its biochemical activity in HPr(Ser-P) dephosphorylation demonstrated. Moreover, a *prpC* mutant strain was isolated and found to be impaired in HPr(Ser-P) dephosphorylation. Homologues of PrpC are present in many bacteria possessing HPr(Ser-P), suggesting that PrpC may play an important role in adjusting the cellular HPr phosphorylation state and thus controlling the diverse regulatory functions exerted by the different forms of HPr.

Bacteria possess highly sophisticated signal transduction systems to survey the nutrient supply in their environment and to respond appropriately. For carbon metabolism, the phosphotransferase system (PTS) has functions comparable with a central processing unit in many bacteria. Small PTS proteins that serve as cofactors for the pleiotropic transcription factor CcpA (5). In addition, HPr(Ser-P) can only be dephosphorylated by the action of the HPr kinase itself, which also exhibits a phosphorylase activity, depending on the presence or absence of easily metabolizable carbon sources. In contrast to the more common protein phosphatases, the phosphorylase transfers the phosphatase. The phosphoryl group is derived from phosphoenolpyruvate and is transferred via enzyme I, HPr, and the sugar-specific permease to the incoming sugar (1). In addition to its function in carbohydrate transport, the PTS is one of the major regulatory systems in many bacteria. This is because of the different phosphorylation state of PTS proteins in the presence or absence of sugars. In *Escherichia coli* and other enteric bacteria, the IIA domain of the glucose permease is the key player in signal transduction mediating either inducer exclusion or the stimulation of cyclic AMP synthesis. In contrast, in Gram-positive bacteria with a low GC content in their chromosomal DNA (i.e. the Firmicutes) as well as in spirochetes and many proteobacteria, the HPr protein plays the central role in the regulation of carbon metabolism (2).

In the Firmicutes, including *Bacillus subtilis*, *Listeria monocytogenes*, and *Mycoplasma pneumoniae*, HPr is not only phosphorylated in a phosphoenolpyruvate-dependent manner on His-15 but is also subject to a regulatory phosphorylation by the metabolite-controlled HPr kinase/phosphorylase (HPrK/P) on Ser-46. Although HPr(His~P~) is required for sugar transport, both HPr(His~P~) and HPr(Ser-P) play distinct roles in the regulation of carbon metabolism and virulence. HPr(His~P~) can phosphorylate several transcription regulators and enzymes, thereby stimulating their activity (3, 4). Moreover, HPr(His~P~) seems to be required for the activity of the *L. monocytogenes* virulence transcription factor PrfA (5). On the other hand, HPr(Ser-P) serves as a cofactor for the pleiotropic transcription factor CcpA that mediates carbon catabolite repression and activation in the Firmicutes (6, 7). The phosphorylation state of HPr depends on the nutrient supply of the bacteria. In the absence of glucose, free HPr and HPr(His~P~) are present in the cells. In contrast, a significant portion of HPr is phosphorylated on Ser-46 when the bacteria grow in the presence of glucose (8, 9). In *B. subtilis*, the two phosphorylation events are mutually exclusive. Once formed, HPr(His~P~) can be dephosphorylated by the transfer of the phosphate group back to enzyme I, to any of a large set of sugar permeases, or to one of the regulatory protein targets. In contrast, HPr(Ser-P) can only be dephosphorylated by the action of the HPr kinase itself, which also exhibits a phosphorylase activity, depending on the presence or absence of easily metabolizable carbon sources. In contrast to the more common protein phosphatases, the phosphorylase transfers the...
phosphate group to an inorganic phosphate, thus generating pyrophosphate (10, 11).

We are interested in the regulatory mechanisms of carbon metabolism in *M. pneumoniae*. These pathogenic bacteria are characterized by their extremely reduced genomes with only a handful of regulatory proteins (12). Projects to create artificial life, the so-called minimal genome concept, did recently attract much scientific interest to the investigation of *Mycoplasma genitalium, M. pneumoniae* and other related cell wall-less bacteria collectively called Mollicutes (13). One of the few regulatory proteins of *M. pneumoniae* is the HPr kinase/phosphorolase (HPrK/P) encoded by the *hprK* gene (14). Unlike its homologue from *B. subtilis*, which exhibits kinase activity only in the presence of high ATP concentrations or when fructose-1,6-bisphosphate is present, the *M. pneumoniae* enzyme is active as a kinase at very low ATP concentrations because of its high affinity for ATP (15). This feature may reflect the adaptation of *M. pneumoniae* to nutrient-rich human mucosal surfaces (14). The structure of the *M. pneumoniae* HPrK/P has been elucidated (Protein Data Bank code 1KNX); however, the reason for the different control of activities as compared with the homologous enzymes from other organisms has so far remained obscure (16, 17). Assays of *in vivo* HPr phosphorylation have revealed that HPr is phosphorylated on His-15 but not on Ser-46 when the bacteria grows with glucose or fructose. HPr(Ser-P) was detectable only in the presence of glycerol (18). This finding is in contrast to the previous biochemical analysis of *M. pneumoniae* HPrK/P and still awaits an explanation. Moreover, a substantial portion of HPr is doubly phosphorylated in the presence of glycerol, suggesting distinct interaction properties of the proteins involved in HPr phosphorylation (19).

The genetic analysis of *M. pneumoniae* is hampered by the lack of a genetic system. Transposons have been used to obtain mutants (13, 20); however, it has so far not been possible to isolate any predetermined desired mutant strains.

In this work, we have described a simple screen for the isolation of *M. pneumoniae* mutants. The analysis of an *hprK* mutant revealed the presence of an additional enzyme involved in the dephosphorylation of HPr(Ser-P). The corresponding gene *prpC* (MPN247) was identified, and the activity of the encoded protein phosphatase was proven *in vitro* and *in vivo*.

**EXPERIMENTAL PROCEDURES**

**Bacterial Strains, Oligonucleotides, and Growth Conditions**—*E. coli* DH5α was used for overexpression of recombinant proteins. The cells were grown in LB medium containing ampicillin (100 µg ml⁻¹). The *M. pneumoniae* strains used in this study were *M. pneumoniae* M129 (American Type Culture Collection 29342) in the 33rd broth passage and its isogenic mutant derivatives GPM51 (*hprK::mini-Tn, Gm⁶*) and GPM68 (*prpC::mini-Tn, Gm⁶*). The oligonucleotides used in this study are listed in Table 1. *M. pneumoniae* was grown at 37 °C in 150-cm² tissue culture flasks containing 100 ml of modified Hayflick medium as described previously (18). Carbon sources were added as indicated. Strains harboring transposon insertions were cultivated in the presence of 80 µg/ml gentamicin.

**DNA Manipulation and Plasmid Construction**—Transformation of *E. coli* and plasmid DNA extraction was performed using standard procedures (21). Enzymatic DNA manipulations and modifications were done as described previously (9). For the amplification of a C-terminally truncated *hprK* allele, the oligonucleotides KS9 and SH30 were used. The PCR fragment was digested with Sall and Nhel and ligated into the overexpression vector pWH844 (22) cut with the same enzymes. The resulting plasmid was sequenced and named GPM66. To overexpress *M. pneumoniae* Prp (MPN247), we constructed plasmid pGP370 in two steps. First, a 1.5-kb fragment containing the *M. pneumoniae* MPN247 gene was amplified from chromosomal DNA using the primers SH64 and SH65. As the MPN247 gene contains a TGA codon that codes for tryptophan (23), the oligonucleotides SH66 and SH67 were used. The MPN247 gene contains a TGA codon that codes for tryptophan in *M. pneumoniae* but for an opal codon in *E. coli*, the PCR fragment was the template for mutagenesis by the combined chain reaction (23) using the amplification primers SH66 and SH67 and the 5¹-phosphorylated mutagenic primer SH68 to introduce an A375G transition. The resulting fragment was cloned between the Sall and Nhel sites of pWH844. The replacement of the TGA by a TGG codon was verified by DNA sequencing.

**Electroporation of M. pneumoniae**—*M. pneumoniae* was transformed with plasmid DNA by electroporation as described previously (24). Transposants were selected on PPLO agar containing 80 µg/ml gentamicin, and single colonies were transferred into modified Hayflick medium also containing 80 µg/ml gentamicin.

**Southern Blot Analysis**—For the preparation of *M. pneumoniae* chromosomal DNA, cells of a 100-ml culture were harvested as described previously (18). The cell pellet was resuspended in 750 µl of 50 mM Tris/HCl, pH 8.0, and 25 mM EDTA, and RNase A was added to a final concentration of 25 µg/ml. After an incubation step at 37 °C for 15 min, 50 µl of proteinase K (25 mg/ml) and 75 µl of 10% SDS were added. The mixture was incubated at 50 °C until the lysate was clarified and subsequently cooled down on ice. To precipitate debris, 300 µl of 5 M NaCl were added, and the mixture was incubated for 20 min on ice. The precipitate was pelleted by centrifugation (25 min, 15000 × g, 4 °C), and the resulting supernatant was mixed with

| TABLE 1 |
| Oligonucleotides used in this study |
|---|
| Oligonucleotide | Sequence (5'→3')<sup>a</sup> |
| KS9 | AAAATGCACTGATGAAAAAGTTATTGCAAGGGAG |
| KS10 | ATTAATGTTGTCTGACTACATATCTAGATTCATC |
| SH3 | GATACCCGCGATTAAAGGG |
| SH4 | CTATAGGACTGTAACATATAGGGAGAAGGAGGACACATATGAGAT |
| SH29 | ATGGATGCTGAGCAATCACAG |
| SH30 | CAATACCGAACACCCCTC |
| SH62 | TAGAATTTTATGCTGATAGG |
| SH63 | CTATAAGCACCTCACTATAGGGAGAAGGAGGACACATATCAG |
| SH64 | GCCTTAGTGGCGAATATCC |
| SH65 | CTGCCTCAGTGGTGTGCA |
| SH66 | AAATCCGACATGGAACACCAACACCAACAC |
| SH67 | AAACATGGCTGACGCTATTATTTAATGAAATCCAAG |
| SH68 | P→GAAACATTTGCGACGTTCGGO |
| SH73 | CTAATACGACCTCACTATAGGGAGAAGGACACATATGAGGACACATATCAG |

<sup>a</sup> The “P” at the 5’-end of the oligonucleotide sequences indicates phosphorylation. The sequence of the T7 promoter is underlined in SH4, SH63, and SH73.
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Isolation of a M. pneumoniae hprK Transposon Insertion Mutant

A schematic drawing of the genomic region next to the hprK gene in M. pneumoniae and site of the transposon insertion in the hprK knock-out strain GPM51. The annealing sites of oligonucleotides used in sequencing reactions for the determination of the transposon insertion site are indicated by arrows. Probes hybridizing to internal fragments of the hprK and the aac-ahpD genes are depicted as dotted lines. B, Southern blot to confirm the single insertion of the mini-transposon into the hprk gene of strain GPM51. Chromosomal DNA of the wild type (wt) and strain GPM51 was digested using NcoI and SmaI. Blots were hybridized with the hprK-specific probe (left) and a probe hybridizing to the aac-ahpD gene of the mini-transposon (right). DIG-labeled DNA molecular weight marker III (Roche Applied Science) served as a standard. C, DNA sequence in the immediate vicinity of the transposon insertion site in strain GPM51. SH30 and SH29 (see Fig. 1A) were used as sequencing primers. The 26-bp-long inverted repeats of the mini-transposon are boxed, and the 8-bp target duplications are underlined.

Western Blot Analysis—In vivo HPr phosphorylation was assayed by Western blot analysis as described previously (18). The different forms of HPr were detected using antibodies directed against M. pneumoniae HPr.

Protein Purification—His6-HPr, His6-HPrK/P, His6-PrpC, and the His6-tagged version of the C-terminally truncated HPrK/P were overexpressed using the expression plasmids pGP217 (14), pGP204 (14), pGP370, and pGP366, respectively. Expression was induced by the addition of isopropyl 1-thio-β-D-galactopyranoside (final concentration 1 mM) to exponentially growing cultures, and the proteins were purified using a Ni2+-nitrilotriacetic acid superflow column as described previously (19). For the recombinant HPr protein, the overproduced protein was purified from the pellet fraction of the lysate by urea extraction and renatured as described previously (14).

In Vitro Activity Assays of HPrK/P and of HPr(Ser-P)-Dephosphorylating Enzymes—HPrK/P activity assays in cell extracts and the preparation of HPr(Ser-P) were carried out as described previously (18, 19). To detect HPr(Ser-P) phosphatase activity in cell extracts, a 20 μM concentration of HPr(Ser-P) was incubated with 10 μg of cellular protein in 25 mM Tris/HCl, 10 mM MgCl2, and 10 mM dithiothreitol in a total volume of 20 μl for 2 h at 37 °C followed by thermal inactivation (19). HPr(Ser-P) phosphatase activity of PrpC was assayed in 20 μl of buffer containing 75 mM Tris/HCl, pH 7.5, 1 mM MnCl2, 1 mM dithiothreitol with 20 μM HPr(Ser-P) and 300 nM His6-PrpC. The dephosphorylation reaction was allowed to proceed for 15 min and stopped immediately by thermal denaturation for 10 min at 95 °C. The assay mixtures were analyzed as described previously (19).

RESULTS

Isolation of a M. pneumoniae hprK Mutant—M. pneumoniae can be subjected to transposon mutagenesis using delivery vectors such as pMT85 (25). We have designed a strategy, designated “haystack mutagenesis,” to isolate any viable desired mutant. This is based on an ordered collection of M. pneumoniae PrpC toward p-nitrophenyl phosphate (PNPP) was assayed in a buffer containing 300 mM Tris/HCl, pH 7.5, 1 mM MnCl2, 1 mM dithiothreitol with 25 mM PNPP, and 5 μg of purified His6-PrpC in a total reaction volume of 1 ml. The reaction was started by the addition of PrpC, carried out for 10 min at 30 °C, and stopped by the addition of 100 μl of 0.1 M EDTA, pH 8.0. The reaction product p-nitrophenol was quantified photometrically at a wavelength of 420 nm.

RESULTS

Isolation of a M. pneumoniae hprK Mutant—M. pneumoniae can be subjected to transposon mutagenesis using delivery vectors such as pMT85 (25). We have designed a strategy, designated “haystack mutagenesis,” to isolate any viable desired mutant. This is based on an ordered collection of M. pneumoniae transposon mutants and the assumptions that these bacteria contain ~200 non-essential genes and that ~920 clones are required to find a desired mutant at a confidence level of 99%. We isolated 2976 individual transposon mutants and grouped them into pools of 50 clones. With this number of mutants, a hprK mutant is included in the library with a probability of 99.999%. Cells of each pool were used in a PCR to detect the occurrence of products corresponding to junctions between the hprK gene and the mini-transposon using the oligonucleotides KS10 and SH29 (see Fig. 1A). From one pool that gave a positive signal, colony PCR with the 50 individual
Identification of PrpC as a Novel Protein Phosphatase That Targets HPr(Ser-P)—Two possible reasons for the phosphatase activity observed in *M. pneumoniae* GPM51 could be: (i) the truncated HPrK/P present in this strain could have still had phosphatase activity or (ii) another protein in *M. pneumoniae* could have been active in HPr(Ser-P) dephosphorylation. To distinguish between these possibilities, we cloned a truncated hprK allele that was identical to the truncated hprk present in GPM51. This protein was purified and used to assay phosphatase activity using HPr(Ser-P) as a substrate. However, although the full-length protein dephosphorylated HPr(Ser-P), no activity was detected using the truncated protein (data not shown). This observation suggests that another protein encoded by *M. pneumoniae* might dephosphorylate HPr(Ser-P).

Dephosphorylation of HPr(Ser-P) by a protein different from HPrK/P has, so far, not been reported in any bacterium. A candidate for such a phosphatase is the protein encoded by the open reading frame MPN247, which is annotated as a PP2C-like protein phosphatase (12). Because phosphatases of this family dephosphorylate a broad range of protein substrates (26, 27), we considered the possibility that the MPN247 gene product was the phosphatase in question. To test this idea, the MPN247 gene was cloned in a way that allowed the subsequent purification of the His-tagged gene product. The fusion protein was purified by affinity chromatography and its activity as a HPr(Ser-P) phosphatase tested. As shown in Fig. 3, complete HPr(Ser-P) dephosphorylation was observed in the presence of manganese ions. Thus, the protein encoded by the MPN247 gene exhibited HPr(Ser-P) phosphatase activity. Based on the similarity of the deduced protein with the *B. subtilis* phosphatase PrpC and on the similar genetic arrangement (clustering with a protein Ser/Thr kinase) (28), the MPN247 gene was renamed *prpC*. An alignment of the *M. pneumoniae* PrpC protein with other phosphatases of the PP2C family is shown in supplemental Fig. S1. As can be seen, the active

\[ \text{HPr(Ser-P)} \]
sites involved in the binding of metal ions and phosphate are highly conserved in all proteins of the family.

**Control of PrpC Activity**—Protein phosphatases of the 2C family are regulated by a broad range of different metabolites, among them inorganic phosphate and glycerol-2-phosphate (28, 29). The regulation of *M. pneumoniae* PrpC was studied using the synthetic substrate PNPP or HPr(Ser-P). First, we determined the kinetic parameters of PrpC activity with PNPP. The *Km* and *Vmax* values were found to be 1.14 ± 0.19 mM and 2.41 ± 0.69 μmol min⁻¹ mg⁻¹, respectively (using a molar extinction coefficient ε₂₄₀ of 12,500 M⁻¹ cm⁻¹). In the presence of inorganic phosphate, the PrpC activity was strongly inhibited in a competitive manner (*Kᵢ* 62 ± 18 μM), whereas glycerol-2-phosphate caused a weak inhibition (50% inhibition at 34 ± 11 mM) (see Fig. 4A). In contrast, glycerol did not affect PrpC activity. The inhibition of PrpC activity by inorganic phosphate and glycerol-2-phosphate was also observed using the natural substrate HPr(Ser-P) (see Fig. 4B).

**HPr Phosphorylation in a prpC Mutant Strain**—To confirm the biological role of PrpC, we isolated a *prpC* mutant from the mutant library using the oligonucleotides SH67 and SH29 as described for the *hprK* mutant. The transposon insertion was verified by Southern blot analysis with probes specific for *prpC* and *aac-ahpD* to demonstrate disruption of the *prpC* region and the unique insertion of the transposon, respectively. The insertion occurred after the 167th nucleotide of *prpC*, giving rise to a truncated protein (see supplemental Fig. S2). The resulting strain was designated GPM68.

To test the HPr(Ser-P) phosphatase activity of the *prpC* mutant strain GPM68, HPr(Ser-P) was incubated in the presence of a cell extract of this strain. As shown in Fig. 2B, almost no HPr dephosphorylation was detected in the *prpC* mutant strain, whereas the phosphatase activity was present both in the wild type and *hprK* mutant strains. The residual HPr(Ser-P) dephosphorylating activity seen with cell extracts of the *prpC* mutant is probably caused by the presence of a functional HPrK in this strain. However, as HPrK absolutely requires phosphate to be active in dephosphorylation of HPr(Ser-P) (14), this activity is rather weak because no additional phosphate was included in this assay. This finding suggests that PrpC might be the major player controlling HPr(Ser-P) dephosphorylation.

The finding that PrpC is a crucial factor in the control of HPr phosphorylation was supported by an analysis of the *in vivo* HPr phosphorylation state. In the presence of glucose, fructose, or glucose and fructose, no HPr(Ser-P) was detectable in the wild type and *prpC* mutant strains. In contrast, HPr(Ser-P) was formed in the presence of glycerol irrespective of the availability of glucose. This is in good agreement with our previous observation that glycerol triggers HPr(Ser-P) formation *in vivo* (18). When both glycerol and glucose were present in the medium, a larger portion of HPr was present in the doubly phosphorylated form and as HPr(Ser-P) in the *prpC* mutant GPM68, as compared with the isogenic wild type strain (Fig. 5). Thus, PrpC is indeed implicated in the regulation of HPr phosphorylation in living cells of *M. pneumoniae*.

**DISCUSSION**

*M. pneumoniae*, *M. genitalium*, and other Mollicutes have recently attracted much interest because of their small genomes and the possibility of defining the minimal genetic equipment required for independent life. However, although random transposon mutageneses suggested sets of genes that might be essential or not (13, 30), it has, so far, not been possible to test these hypotheses by the directed isolation of mutants. The haystack mutagenesis approach developed in this work allowed us to obtain any desired viable mutant in an easy way. Our results confirm the previ-
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![Image of protein phosphorylation pattern](image)

**FIGURE 5.** In vivo HPr phosphorylation pattern in the M. pneumoniae prpC mutant GPM68. Protein extracts of cultures of the wild type (wt) and the GPM68 strain that had been grown in the presence of glucose and glycerol to induce HPr(Ser-P) formation were subjected to native gel electrophoresis and electroblotted onto a polyvinylidene difluoride membrane. The membrane was detected using a polyclonal rabbit antiserum against M. pneumoniae HPr. For the discrimination of both singly phosphorylated HPr forms, a parallel aliquot of each sample was heat-exposed (10 min, 70 °C) to hydrolyze the heat-labile HPr(His–P).

ous finding that the hprK gene is non-essential, but they contradict the assignment of prpC (MPN247) as one of the essential genes (13, 30). With the transposon mutant library at hand, the essentiality of each of the predicted genes can easily be verified or eliminated. Moreover, with the availability of a tool for mutant isolation, the research on *M. pneumoniae* and related species will be significantly accelerated, making thus far intractable problems accessible for investigation.

The reductive evolution of *M. pneumoniae* has resulted in the loss of nearly all regulatory genes. Among the few regulatory responses observed in *M. pneumoniae* are the induction of heat shock genes upon temperature upshift and the phosphorylation of HPr on Ser-46 in the presence of glycerol (18, 31). The formation of HPr(Ser-P) is catalyzed by the HPrK/P encoded by the *hprK* gene (14), and this is the only enzyme in *M. pneumoniae* with such an activity. In contrast, dephosphorylation of HPr(Ser-P) is not exclusively catalyzed by HPrK, as detected with such an activity. In contrast, dephosphorylation of HPr(Ser-P) is catalyzed by the HPrK/P encoded by the *hprK* gene (14), and this is the only enzyme in *M. pneumoniae* with such an activity. In contrast, dephosphorylation of HPr(Ser-P) is not exclusively catalyzed by HPrK, as detected using the *hprK* mutant strain. Surprisingly, an additional enzymatic activity was detected, and we showed here that the protein phosphatase PrpC is responsible for this activity. Thus, a novel protein is implicated in controlling the phosphorylation state of HPr. Enzyme I of the PTS phosphorylates His-15 of HPr, and this phosphate residue can be transferred to either of the two functional PTS sugar permeases, i.e. the glucose permease or the fructose permease (18). HPrK/P mediates phosphorylation of Ser-46, but both HPrK/P and PrpC catalyze dephosphorylation of HPr(Ser-P). The presence of two different enzymes for this purpose has interesting implications. The phosphatase activity of HPrK/P is triggered when the concentration of inorganic phosphate is high in the cell (14, 15). It has been suggested that these conditions occur when the cells are depleted of nutrients (32). In contrast, PrpC is strongly inhibited by the presence of inorganic phosphate (see Fig. 4). In summary, the intracellular phosphate concentration does not seem to be important for the dephosphorylation of HPr(Ser-P), because one of the two enzymes is active under either condition.

The HPr phosphorylation state is of key importance for the control of carbon metabolism in the Firmicutes. HPr can either serve in sugar transport, it can activate transcriptional regulators and enzymes, or it can be a co-factor of a transcriptional regulator. In the model organism *B. subtilis*, HPr is phosphorylated on Ser-46 when the bacteria grow on glycolytically metabolizable carbon sources such as glucose (8, 9). It has long been believed that HPrK/P is the only protein phosphorylating HPr or dephosphorylating HPr(Ser-P). Interestingly, only part of HPr is phosphorylated on Ser-46, even when the bacteria grow in the presence of glucose. In contrast, all HPr was converted to HPr(Ser-P) in a mutant strain devoid of the transcriptional regulator CcpA (9). From these data, it was concluded that additional factors might control HPr phosphorylation. It is tempting to speculate that PrpC is the protein for which we are searching. Indeed, PrpC is also present in *B. subtilis*. In the presence of glucose (low phosphate), it might dephosphorylate a part of HPr(Ser-P), which remains available for sugar transport.

PrpC is a member of the family of PP2C protein phosphatases. These enzymes use a broad spectrum of phosphorylated substrates, including the artificial substrate PNPP, the PII protein in cyanobacteria (33), or anti-α factors and the translation factor EF-G in *B. subtilis* (34, 35). It will be interesting to analyze the molecular interactions between HPr(Ser-P) and PrpC as well as the physiological roles of this phosphatase in *M. pneumoniae* as well as in other bacteria that possess HPr(Ser-P).

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