The PrpC Serine-Threonine Phosphatase and PrkC Kinase Have Opposing Physiological Roles in Stationary-Phase *Bacillus subtilis* Cells

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Received 18 December 2001/Accepted 19 August 2002

Loss of the PrpC serine-threonine phosphatase and the associated PrkC kinase of *Bacillus subtilis* were shown to have opposite effects on stationary-phase physiology by differentially affecting cell density, cell viability, and accumulation of β -galactosidase from a general stress reporter fusion. These pleiotropic effects suggest that PrpC and PrkC have important regulatory roles in stationary-phase cells. Elongation factor G (EF-G) was identified as one possible target of the PrpC and PrkC pair in vivo, and purified PrpC and PrkC manifested the predicted phosphatase and kinase activities against EF-G in vitro.

Analysis of signaling proteins encoded in complete bacterial genomes has revealed a wide distribution for serine-threonine kinases and phosphatases (22), yet the regulatory functions for many of these are unknown. *Bacillus subtilis*, with its four described serine-threonine kinases (1, 7, 15, 26) and five described phosphatases (8, 18, 25, 26), provides a good model to study the role of serine-threonine phosphorylation in prokaryotes.

Three of these kinases (RsbT, RsbW, and SpoIIAB) and four of the phosphatases (RsbP, RsbU, RsbX, and SpoIIE) act via a partner-switching mechanism to control the activities of the general stress transcription factor σ^B and the forespore-specific transcription factor σ^F (reviewed in reference 19). The three partner-switching kinases are unusual in that their sequences reflect more of a kinship with bacterial histidine protein kinases than with typical eukaryotic serine-threonine kinases (9, 15). The four partner-switching phosphatases all belong to the PPM/PP2C family, but they are also unusual in that they lack conserved domains V_a and V_b that are commonly found in eukaryotic PP2C phosphatases (22).

In contrast, the PrpC phosphatase and the PrkC kinase more closely resemble their eukaryotic counterparts, but their physiological roles are unknown (18, 22). Because it is thought that at least one serine-threonine phosphatase in the σ^B signal transduction network remains to be discovered (10), we sought to determine the effects of loss-of-function mutations within *prpC* and *prkC*. Given the multiple phenotypes elicited by these mutations, we infer that PrpC and PrkC have a significant role in controlling a variety of stationary-phase processes.

MATERIALS AND METHODS

Bacterial strains and genetic methods. Standard recombinant DNA methods and transformation of *B. subtilis* strains were as previously described (10). We made in-frame deletions in the *prpC* and *prkC* reading frames using the four-primer method of site-directed mutagenesis (11) and we substituted these for the wild-type alleles by a two-step replacement procedure (23). Strain PB703

(*prpC* Δ 1) bore a deletion of triplets 18 to 224 within *prpC*; strain PB706 (*prkC* Δ 1) bore a deletion of triplets 33 to 611 within *prkC*; and strain PB723 (*prpC*-*prkC* Δ 1) bore a deletion extending from triplet 18 of *prpC* to triplet 611 of *prkC*. These strains also carried a single-copy transcriptional fusion between the σ^B -dependent *ctc* promoter and a *lacZ* reporter gene in order to permit comparison to strain PB198, which has the same fusion but is wild type at the *prpC* and *prkC* loci (6).

β -Galactosidase accumulation assays. For the experiments shown in Table 1, cells were grown into stationary phase in buffered Luria broth (LB) lacking salt (BLB) (5). For environmental stress experiments (data not shown), cells were grown to early logarithmic phase in BLB, at which point NaCl was added to a 0.3 M final concentration. For both assays samples were collected and treated as described by Miller (14). Cells were washed with Z buffer and permeabilized using sodium dodecyl sulfate (SDS) and chloroform. Protein levels were determined using the Bio-Rad protein assay reagent (Bio-Rad Laboratories, Richmond, Calif.). Activity was defined as $\Delta A_{420} \times 1,000$ per minute per milligram of protein.

Western blotting experiments. Wild-type and mutant cultures were grown in BLB, harvested in logarithmic phase (50 Klett units in a Klett-Summerson colorimeter, using a number 66 filter), and then resuspended in sonication buffer (50 mM Tris, pH 7.5; 50 mM MgCl₂). Cells were broken by sonication, and extracts were prepared by centrifugation. Proteins were separated on SDS-polyacrylamide gels and then transferred to polyvinylidene difluoride membranes (Bio-Rad Laboratories). These blots were blocked and washed in membrane blocking solution (Zymed Laboratories, San Francisco, Calif.) that was compatible with the primary rabbit antibody, either antiphosphothreonine (catalog no. 71-8200; Zymed Laboratories) or antiphosphoserine (catalog no. 61-8100). Blots were exposed to 1 μ g of primary antibody/ml in TBS-T buffer (20 mM Tris, pH 7.6; 137 mM NaCl; 0.1% Tween 20) for 1 h at 25°C and then to the anti-rabbit immunoglobulin G (IgG) peroxidase-conjugated secondary antibody (Sigma, St. Louis, Mo.). Blots were washed, and the bound antibody was visualized using the ECL Plus Western blotting detection kit (Amersham Pharmacia Biotech, Piscataway, N.J.), according to the manufacturer's instructions.

Immune precipitation experiments. We did two different types of immune precipitation experiments. The first was an immune precipitation from cell extracts, in which cells were grown and extracts made as described for the Western blotting. Here we began with a 150-ml culture, resulting in 4 ml of extract in sonication buffer. To this we added 9 μ l of anti-*Escherichia coli* elongation factor G (EF-G) antibody, the generous gift of Andreas Savelsbergh and Wolfgang Wintermeyer. After incubating for 1 h on ice, we added 300 μ l of protein A beads (Sigma) suspended in IP buffer (50 mM Tris, pH 8.0; 150 mM NaCl; 0.1% SDS) and then continued the incubation for 1 h at 4°C with slow shaking. The beads were collected by centrifugation, washed three times with IP buffer, and suspended in 150 μ l of Laemmli sample buffer (60 mM Tris, pH 6.8; 100 mM dithiothreitol [DTT]; 2% SDS; 10% glycerol; 0.001% bromophenol blue). After heating at 85°C for 10 min, samples were loaded onto a polyacrylamide gel for analysis by Coomassie staining and Western blotting (see Fig. 2B). The second type of immune precipitation was to purify ³²P-labeled EF-G from in vitro kinase reactions. Here, we added 5 μ l of anti-*E. coli* EF-G antibody to 210 μ l of kinase

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TABLE 1. Loss of PrpC or PrkC function has multiple effects on late-stationary-phase cells^a

Relevant genotype	Cell density (Klett units) ^b	Viability (CFU/ml, mean $\pm$ SD) ^c	β -Galactosidase accumulation ^d
Wild type	70 $\pm$ 16	(1.7 $\pm$ 0.9) $\times$ 10 ⁸	451 $\pm$ 159
<i>prkC</i> Δ 1	25 $\pm$ 9	(1.4 $\pm$ 1.2) $\times$ 10 ⁷	54 $\pm$ 14
<i>prpC</i> Δ 1	347 $\pm$ 25	(2.5 $\pm$ 0.4) $\times$ 10 ⁸	575 $\pm$ 156
<i>prpC-prkC</i> Δ 1	27 $\pm$ 10	(1.0 $\pm$ 0.7) $\times$ 10 ⁷	119 $\pm$ 72

^a Samples were taken 23 to 27 h after the onset of stationary phase in BLB medium (5), at the point of lowest cell density for the wild-type strain. Underlined values significantly differ from wild-type value at the 95% confidence level (Student's two-tailed *t* test).

^b Klett units were measured with a Klett-Summerson photoelectric colorimeter, with a number 66 (red) filter.

^c Determined by standard plate count.

^d From the σ^B -dependent *ctc-lacZ* transcriptional fusion borne by these strains

reaction mix (see below). After incubating for 1 h on ice, we added 100 μ l of protein A beads suspended in IP buffer and then continued the incubation for 1 h at 4°C with slow shaking. The beads were collected by centrifugation and then washed three times with IP buffer and three times with kinase buffer lacking DTT (see below for kinase buffer). The beads were resuspended in 300 μ l of phosphatase buffer (see below), of which half was used to monitor the immune precipitation (see Fig. 3B) and half was used for the phosphatase release assay.

Kinase and phosphatase assays. We constructed overexpression clones which fused the *fus* (EF-G), *prpC*, and *prkC* coding regions to the hexahistidine tag in the pET15b expression vector (Novagen, Madison, Wis.). Tagged proteins were purified from *E. coli* BL21(DE3) extracts on nickel affinity columns by using the manufacturer's protocol (Novagen). The phosphatase and kinase rapidly lost activity when stored in glycerol at -20°C , so all assays were done with freshly purified proteins. For kinase assays, 5 μ g of PrkC and 5 μ g of EF-G (final concentration, 2 μM each) were incubated at 37°C for 30 min in 30 μ l of kinase buffer (50 mM Tris, pH 7.6; 50 mM KCl; 10 mM MgCl₂; 1 mM DTT; 0.1 mM EDTA) together with 1 mM unlabeled ATP and 15 μCi of [γ -³²P]ATP (Perkin Elmer Life Sciences, Boston, Mass.). This kinase reaction mixture was also used to prepare labeled substrate for the phosphatase assays. For the assay shown below in Fig. 3A, PrkC and EF-G were labeled in the kinase reaction, separated from unincorporated [γ -³²P]ATP on a nickel affinity column, resuspended in 30 μ l of phosphatase buffer (kinase buffer plus 2 mM MnCl₂), and then incubated at 37°C with 2 μ g of purified PrpC (2 μM final concentration) for the times indicated. Reactions were terminated by heating in Laemmli sample buffer, and the proteins were analyzed by SDS-polyacrylamide gel electrophoresis (SDS-PAGE). The phosphatase assay shown below in Fig. 3C was done essentially as described previously (25). Immune-precipitated, labeled EF-G in 150 μ l of phosphatase buffer was incubated at 37°C together with 5 μ g of purified PrpC (1 μM final concentration). Samples were removed at the times indicated, treated with cold trichloroacetic acid, and centrifuged. The supernatants were counted in a scintillation counter to determine the amount of γ -³²P released.

In-gel digestion and mass spectrometry. The band containing the protein of interest was excised from a 6% polyacrylamide gel, washed with Milli-Q water (Millipore, Bedford, Mass.), and diced, and the protein was reduced and alkylated (21). The protein was digested in 50% ammonium bicarbonate containing sequence-grade, modified trypsin (Promega, Madison, Wis.). After extraction with 0.1% trifluoroacetic acid and with 5% formic acid in 50% acetonitrile, peptide mass mapping was done using a Bruker Biflex III matrix-assisted laser desorption/ionization-time-of-flight (TOF) mass spectrometer (Bruker-Franzen Analytik, Bremen, Germany) equipped with a pulsed N₂ laser (337 nm), a delayed extraction ion source, and a reflectron. Mass spectra were acquired in the reflectron mode. Internal mass calibration was performed with two trypsin-autodigested fragments, and then measured monoisotopic masses of the tryptic peptides from the protein of interest were used to search international databases. A mass accuracy of 50 ppm or better was used for each search. De novo sequencing of the tryptic peptides by tandem mass spectrometry (MS/MS) was done using hybrid nanospray-electrospray ionization-Quadrupole-TOF-MS and MS/MS in a QSTAR mass spectrometer (Allied Biosystems, Inc., Foster City, Calif.). QSTAR instrument calibration was via a standard peptide mixture and routinely gave mass accuracies of 5 ppm or better. De novo sequencing of peptides was done using QSTAR software and confirmed by manual interpretation of MS/MS spectra.

FIG. 1. Effect of *prpC* and *prkC* alleles on stationary-phase cell density. Cultures were grown at 37°C in shake flasks containing BLB medium; densities were measured at the times indicated using a Klett-Summerson photoelectric colorimeter with a number 66 (red) filter. Cultures entered stationary phase at 2.5 h. Symbols: ○, PB198 wild type; ●, PB703 lacking the phosphatase activity (*prpC* Δ 1); ▲, PB706 lacking the kinase activity (*prkC* Δ 1); ■, PB723 lacking both activities (*prpC-prkC* Δ 1).

RESULTS

Null alleles of *prpC* and *prkC* have opposite stationary-phase phenotypes. *prpC* and *prkC* lie adjacent on the chromosome and appear to comprise part of a multigene operon (12). To learn the physiological role of their products, we made large, in-frame deletions in *prpC*, *prkC*, or both. For wild-type and mutant strains, we then assayed (i) growth rate in a minimal glucose medium (3) and in buffered LB (BLB) lacking salt (5); (ii) σ^B activity upon salt stress or entry into stationary phase (5, 6); and (iii) sporulation in double-strength Schaeffer's medium (13). None of the mutant strains differed from wild type under these conditions. We conclude that the PrpC phosphatase and PrkC kinase activities are not essential for growth or sporulation under standard laboratory conditions. We also conclude that PrpC and PrkC are not required for activation of σ^B via either the environmental or energy-stress signaling pathways (19).

In contrast to the absence of an observable phenotype in logarithmic or early-stationary-phase cells, the *prpC* and *prkC* null mutations had large effects on cell density during extended incubation in stationary phase. One such experiment is shown in Fig. 1. In replicate growth experiments in BLB lacking salt, wild-type cell density decreased to a minimum between 23 and 27 h after the onset of stationary phase (Table 1). Notably, the density of the *prkC* null mutant was significantly lower than that of the wild type, whereas the density of the *prpC* null mutant was significantly higher. Similar results were obtained with cells grown in standard LB, but in this medium wild-type density reached a minimum between 30 and 34 h after the onset of stationary phase (data not shown).

We also did a standard plate count to measure cell viability at the point when wild-type cells reached minimum density (Table 1). The observed differences between wild-type and mutant cells were reproducible but not significant at the 95% confidence level. However, microscopic examination showed that the *prpC* mutant formed abundant chains in stationary

phase, suggesting that a standard plate count would greatly underestimate the number of viable cells. In contrast, both the wild-type strain and the *prkC* mutant were found as discrete cells (data not shown).

Interestingly, the significant differences in cell density between wild-type and mutant strains were reflected in β -galactosidase accumulation from the σ^B -dependent *ctc-lacZ* fusion they carried. In particular, accumulation in the *prkC* mutant was significantly less than in the wild type (Table 1). This distinction was not due to differential lysis, because β -galactosidase activity was assayed in intact cells, collected by centrifugation. It therefore appears that σ^B is less active during late stationary phase in the *prkC* mutant, or that the reporter fusion is more labile.

Based on the diverse effects the *prpC* and *prkC* mutations have on stationary-phase physiology (Table 1), we propose that the PrpC phosphatase and PrkC kinase have important regulatory roles in stationary-phase cells. Moreover, the *prkC* null allele is epistatic to the *prpC* null, at least with respect to the three phenotypes we tested. We therefore hypothesize that loss of PrpC phosphatase activity promotes high cell density in stationary phase due to an inability to counter PrkC kinase activity.

Cell extracts of the *prpC* null mutant have elevated levels of a protein recognized by antiphosphothreonine antibody. In order to identify possible protein substrates of the PrpC phosphatase or the PrkC kinase, we separated extracts of wild-type and mutant cells by SDS-PAGE and probed Western blots of these gels with antibodies that specifically recognize either phosphoserine or phosphothreonine. No significant differences were seen using antibody specific for phosphoserine (data not shown). However, using antibody specific for phosphothreonine, the extract of the *prpC* phosphatase mutant displayed a new signal which migrated with an apparent molecular weight of 105,000 (Fig. 2A, lane 3). Because this signal was absent in extracts from the *prkC* kinase mutant and the *prpC-prkC* double mutant, we infer that the PrkC kinase was directly or indirectly required for its appearance in vivo.

Only 56 *B. subtilis* proteins have calculated molecular weights exceeding 100,000 (12; <http://genolist.pasteur.fr/SubtiliList/>). We therefore ran SDS-6% polyacrylamide gels to separate the proteins in the region of interest and were able to associate the antiphosphothreonine signal with a single protein band (data not shown). The band was extracted from the gel and digested with trypsin. The resulting fragments were analyzed by using MS, which identified EF-G with high confidence. A total of 46% (23 of 49) of the theoretical tryptic fragments were identified by mass, and these fragments were distributed over the entire length of the protein. Moreover, the amino acid sequence of these fragments exactly matched the corresponding fragments in EF-G. Therefore, EF-G became a good candidate as a target for the PrpC phosphatase and the PrkC kinase in vivo.

Immune precipitation using anti-*E. coli* EF-G yields a protein recognized by antiphosphothreonine antibody. The calculated molecular weights of EF-G and the PrkC kinase are 76,360 and 71,687, respectively (12). However, in our hands hexahistidine-tagged versions of these two proteins both migrated on SDS-PAGE with apparent molecular weights of between 105,000 and 110,000 (see below). This anomalous mo-

FIG. 2. Detection of an additional phosphorylated protein in the strain lacking PrpC phosphatase activity. (A) Extracts of cells growing logarithmically in BLB medium were subjected to SDS-PAGE. A Western blot made from this 10% gel was probed with antiphosphothreonine antibody. Lane 1, PB198 wild type; lane 2, PB706 lacking the kinase activity (*prkCΔ1*); lane 3, PB703 lacking the phosphatase activity (*prpCΔ1*); lane 4, PB723 lacking both activities (*prpCΔ1 prkCΔ1*). The arrow indicates the new signal present in lane 3. Mobilities of the molecular weight standards are shown on the left (in thousands). (B) SDS-PAGE analysis of the proteins precipitated from *B. subtilis* cell extracts by anti-*E. coli* EF-G antibody. Lanes 1 and 2 were stained with Coomassie blue and each manifested a band with the mobility of *B. subtilis* EF-G. Lane 1, PB703 lacking the phosphatase activity (*prpCΔ1*); lane 2, PB198 wild type. Lanes 3 and 4 were analyzed by Western blotting, and lane 3 manifested a band recognized by antiphosphothreonine antibody (arrow). Lane 3, PB703 lacking the phosphatase activity (*prpCΔ1*); lane 4, PB198 wild type. Mobilities of the molecular weight standards are on the left.

bility was previously observed for native EF-G (4), but the aberrant mobility of PrkC was somewhat surprising. Notably, the PrkC kinase is thought to autophosphorylate on a threonine residue and is also known to be a substrate of the PrpC phosphatase (18). Given the close comigration of EF-G and PrkC in SDS-PAGE, it remains possible that the antiphosphothreonine signal seen in vivo (Fig. 2A) was in fact PrkC itself, present as a minor component in the EF-G band.

To address this issue, we used anti-*E. coli* EF-G antibody to perform an immune precipitation from a cell extract of a PrpC phosphatase mutant. This yielded a protein with the expected mobility of *B. subtilis* EF-G (Fig. 2B, lane 1), and a parallel Western blot using antiphosphothreonine antibody revealed a signal with the same mobility (Fig. 2B, lane 3). This signal was absent in the wild-type control (Fig. 2B, lane 4). Because we show below that the anti-*E. coli* EF-G antibody preferentially

FIG. 3. Purified PrpC phosphatase and PrkC kinase are active against EF-G *in vitro*. (A) Lanes 1 to 3 show the kinase assay. Purified proteins were incubated at 37°C in kinase buffer together with $[\gamma\text{-}^{32}\text{P}]\text{ATP}$ and then separated by SDS-PAGE. Lane 1, PrkC alone; lane 2, EF-G alone; lane 3, PrkC and EF-G. Lanes 4 to 7 show the phosphatase assay. After completion of the kinase reaction, labeled PrkC and EF-G were separated from unincorporated $[\gamma\text{-}^{32}\text{P}]\text{ATP}$, resuspended in phosphatase buffer, incubated at 37°C, and then separated on an SDS-PAGE gel. Lane 4 shows a 60-min incubation in the absence of the PrpC phosphatase; lanes 5 to 7 show 15-, 30-, and 60-min incubations in the presence of purified PrpC. Arrows here and in panel B denote the positions of unlabeled PrkC and EF-G included as standards. (B) Immune precipitation of EF-G from a kinase labeling reaction. Purified proteins were incubated with $[\gamma\text{-}^{32}\text{P}]\text{ATP}$ as described for panel A and then mixed with anti-*E. coli* EF-G antibody and protein A beads. The resulting precipitate was subjected to SDS-PAGE. Lane 1, the kinase reaction yielding labeled PrkC and EF-G; lane 2, the labeled EF-G recovered by immune precipitation; lane 3, a longer exposure of lane 2. (C) Phosphatase release assay. The immune precipitate shown in lanes 2 and 3 of panel B was washed and resuspended in phosphatase buffer and then incubated at 37°C together with PrpC. Samples were removed at the times indicated, treated with cold trichloroacetic acid, centrifuged, and counted to determine the amount of label released into the supernatant fraction. (D) Determination of antibody specificity. Purified PrkC and EF-G were incubated in a standard kinase reaction with 1 mM cold ATP and no labeled ATP. The reaction was terminated, divided into three equal parts, and analyzed by SDS-PAGE and Western blotting with antiphosphothreonine antibody. Lane 1, antibody alone; lane 2, antibody after preincubation with 20 mM phosphothreonine; lane 3, antibody after preincubation with 20 mM phosphoserine. The PrkC and EF-G signals are indicated by arrows.

precipitated EF-G over PrkC (Fig. 3B) and that the antiphosphothreonine antibody was specific for phosphothreonine (Fig. 3D), these results support the hypothesis that *B. subtilis* EF-G is phosphorylated on a threonine residue in a mutant lacking PrpC activity.

PrkC adds a phosphate to EF-G *in vitro* and PrpC removes this phosphate. To determine if EF-G was a substrate for the PrkC kinase and PrpC phosphatase *in vitro*, we his-tagged all three proteins and purified them on nickel affinity columns. As shown in Fig. 3A, incubation of purified PrkC with $[\gamma\text{-}^{32}\text{P}]\text{ATP}$ alone yielded a labeled protein with the mobility of the PrkC standard (lane 1), whereas incubation of purified EF-G produced no labeled protein (lane 2). However, when EF-G was incubated together with PrkC, a second labeled protein with the mobility of the EF-G standard appeared (lane 3). Thus, PrkC is capable of phosphorylating EF-G *in vitro*.

We then used EF-G phosphorylated by PrkC to determine whether EF-G might be a substrate for the PrpC phosphatase. We first incubated EF-G and PrkC proteins in kinase buffer together with $[\gamma\text{-}^{32}\text{P}]\text{ATP}$, and then we purified the labeled proteins on a nickel affinity column to separate them from unincorporated $[\gamma\text{-}^{32}\text{P}]\text{ATP}$. Both labeled proteins were incubated in phosphatase buffer together with the purified PrpC phosphatase. As shown in Fig. 3A (lanes 4 to 7), the presence of PrpC progressively decreased the amount of the labeled PrkC, as expected from the work of Obuchowski et al. (18). However, the presence of PrpC also progressively decreased the amount of labeled EF-G in the same reaction.

The experiment shown in Fig. 3A did not allow us to distinguish between direct dephosphorylation of EF-G by the PrpC phosphatase or indirect dephosphorylation via a reverse reaction involving the PrkC kinase. To address this issue, we first

prepared EF-G phosphate by incubation with PrkC and [γ - 32 P]ATP. We then purified the EF-G phosphate by immune precipitation with antibody specific for *E. coli* EF-G. This resulted in a preparation in which EF-G phosphate was the primary labeled component (Fig. 3B). When this EF-G phosphate preparation was incubated with PrpC phosphatase, the 32 P label was efficiently removed in a phosphate release assay (Fig. 3C). Although we cannot eliminate the possibility that a small amount of PrkC kinase remained in our EF-G phosphate preparation and catalyzed the reverse reaction, we consider it more likely that the PrpC phosphatase directly dephosphorylates EF-G phosphate in vitro.

To determine whether PrkC and EF-G were in fact phosphorylated on a threonine residue in vitro, we performed the Western blotting experiment shown in Fig. 3D. This experiment also served to test antibody specificity. The antiphosphothreonine antibody detected two strong bands at the mobilities expected for phosphorylated PrkC and EF-G (lane 1). Preincubation of this antibody with phosphothreonine effectively competed both these signals (lane 2), whereas preincubation with phosphoserine had little effect (lane 3). Because the antiphosphothreonine antibody had no significant avidity to phosphoserine, we conclude that both PrkC and EF-G are phosphorylated on one or more threonine residues.

DISCUSSION

Disruptions of the adjacent *prpC* and *prkC* genes have pleiotropic effects in stationary-phase cells, suggesting an important regulatory role. The most striking of these effects is the unusually high cell density observed in the *prpC* mutant during late stationary phase. Because loss of PrkC function overrides loss of PrpC function, we hypothesize that the primary role of the PrpC phosphatase is to counter the action of the PrkC kinase. One possible target of the PrpC-PrkC pair in vivo is EF-G, which is a substrate for the PrkC kinase and PrpC phosphatase in vitro.

Activity of EF-2, the EF-G homologue in eukaryotic cells, is known to be dramatically decreased by threonine phosphorylation (reviewed in reference 17). Moreover, *E. coli* EF-G is known to be phosphorylated during phage T7 infection (20), and it was suggested earlier that a *B. subtilis* EF-G homologue becomes phosphorylated during the sporulation process (16). It is therefore an attractive possibility that the dynamic control of the phosphorylation state of EF-G serves a regulatory role in stationary-phase *B. subtilis* cells, and that disruption of this control contributes to the observed phenotypes of the *prpC* and *prkC* mutants. If threonine phosphorylation of bacterial EF-G serves to decrease its activity, as is the case for eukaryotic EF-2, we can imagine that the resulting slow polypeptide extension would redirect free ribosomal subunits to mRNA species that have low initiation rates, primarily as a consequence of decreased competition with mRNA species that have high initiation rates.

PrkC bears a clear serine-threonine kinase domain resembling those found in eukaryotic kinases, and this domain is widely distributed among prokaryotes. However, true orthologs of the full-length PrkC appear to be confined to the low-GC group of gram-positive bacteria (Table 2). This reflects the fact that PrkC has two domains—an amino-terminal kinase

TABLE 2. Representative organisms with linked *prpC* and *prkC* paralog genes

Organism	Similarity to PrpC phosphatase		Similarity to PrkC kinase	
	Accession no.	E value ^a	Accession no.	E value ^a
<i>Listeria monocytogenes</i>	CAC99899	4c-60	CAC99898	4c-159
<i>Streptococcus pyogenes</i>	AAK34397	1e-43	AAK34396	1e-99
<i>Staphylococcus aureus</i>	BAB57381	8e-42	BAB57382	1e-98
<i>Lactococcus lactis</i>	CAA10712 ^b	5e-41	AAK05985 ^c	2e-99
<i>Clostridium acetobutylicum</i>	AAK79693	4e-34	AAK79694	3e-69

^a E-value of BLAST 2.0 comparison (2) using default parameters at the National Center for Biotechnology Information website (<http://www.ncbi.nlm.nih.gov/BLAST/>).

^b Full-length protein. Truncated entry is AAK05986.

^c Full-length protein. Truncated entry is CAA10713.

domain, which is widely shared, and a carboxyl-terminal domain of unknown function, which is of more restricted distribution (24; <http://www.ncbi.nlm.nih.gov/COG/>). Among these low-GC gram-positive bacteria, genes encoding PrpC and PrkC orthologs are directly adjacent, as they are in *B. subtilis*, suggesting that they fulfill equivalent regulatory roles in these organisms.

ACKNOWLEDGMENTS

This research was supported by Public Health Service grant GM42077 from the National Institute of General Medical Sciences.

We thank the two anonymous reviewers for their helpful comments, Young-Moo Lee of the Protein Structure Laboratory for his assistance with the mass spectrometer analysis, and Andreas Savelsbergh and Wolfgang Wintermeyer for providing polyclonal antibody against *E. coli* EF-G.

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