Overexpression of the PepF Oligopeptidase Inhibits Sporulation Initiation in \textit{Bacillus subtilis}\textsuperscript{†}

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The \textit{yjbG} gene encoding the homologue of the PepF1 and PepF2 oligoendopeptidases of \textit{Lactococcus lactis} (Monnet et al., J. Biol. Chem. 269:32070–32076, 1994; Nardi et al., J. Bacteriol. 179:4164–4171, 1997) has been identified in \textit{Bacillus subtilis} as an inhibitor of sporulation initiation when present in the cells on a multicopy plasmid. Genetic analysis suggested that the inhibitory effect is due to hydrolysis of the PhrA peptide in a form as small as the pentapeptide (ARNQT). Inactivation of PhrA results in deregulation of the RapA phosphatase and thus dephosphorylation of the Spo0F–P response regulator component of the phosphorelay for sporulation initiation. When overexpressed, the \textit{B. subtilis} PepF is most likely hydrolyzing additional peptides of the Phr family, as is the case for PhrC involved in control of competence development. Chromosomal inactivation of the \textit{yjbG}/pepF gene did not give rise to any detectable phenotype. The function of PepF in \textit{B. subtilis} remains unknown. Limited experiments with a \textit{yjbG} paralogue called \textit{yusX} indicated that a frameshift is present, making the corresponding gene product inactive.

The initiation of the sporulation process in \textit{Bacillus subtilis} is controlled by a multicomponent signal transduction system termed the phosphorelay (4). The phosphorelay is a more complex version of the typical two-component signal transduction system. Five histidine kinases, KinA to KinE, in response to differential stimuli, activate the system through autophosphorylation and transfer the phosphoryl group to the intermediate response regulator Spo0F (11). By means of the Spo0B phosphotransferase, an enzyme unique to the phosphorelay, the phosphoryl group is then transferred from Spo0F to the Spo0A response regulator, Spo0A is a transcription factor whose activation by phosphorylation triggers the initiation of transcription of developmental genes (8).

The multicomponent structure of the phosphorelay was originally postulated to provide more access points for regulation of phosphate flow in the system (22). A series of negative regulators of sporulation initiation have been identified that directly influence the level of phosphorylation of the phosphorelay components. Autophosphorylation of the major histidine kinase of the system, KinA, is inhibited by the KipI protein (41). Phosphorylated Spo0A is subject to the phosphatase activity of the Spo0E family of response regulator aspartylphosphate phosphatases (22, 28). The phosphorylation level of Spo0F is modulated by three members of the Rap family of phosphatases, RapA, RapB, and RapE (10, 29). RapA and RapE activity is subject to direct inhibition by the PhrA and PhrE pentapeptide inhibitors, respectively (10, 30). These pentapeptide inhibitors arise from an export-import control circuit in which the original products of the \textit{phr} genes (44-amino-acid preinhibitors) are exported by the protein secretion apparatus and are cleaved during the process to pentapeptides that are internalized by the oligopeptide permease transport system (Opp) (26). A failure in the regulatory circuit for PhrA and PhrE pentapeptide production results in derepression of the corresponding Rap phosphatase, with the consequence that sporulation initiation is inhibited (27).

A common feature of negative regulators of sporulation initiation such as KipI, Spo0E, and Rap phosphatases is their ability to inhibit the process when overexpressed from a multicopy plasmid. Here we report the identification of an additional gene, \textit{yjbG}, whose overexpression also results in inhibition of sporulation initiation. \textit{yjbG} encodes a protein highly homologous to the PepF1 and PepF2 oligopeptidases of \textit{Lactococcus lactis} (18, 21). PepFs are cytoplasmic endopeptidases that hydrolyze oligopeptides but cannot degrade proteins, therefore the name oligopeptidases. PepF1 and PepF2 are plasmid- and chromosomally encoded gene products, respectively, of \textit{L. lactis} strain NCD0763. They belong to the M3 family of peptidases that includes the mammalian oligopeptidase 24.15 (17, 32). Oligopeptidases are generally characterized by substrate size specificity. It was shown that PepF1 did not cleave peptides containing fewer than 7 or more than 17 amino acids (18). The substrate size specificity of oligopeptidases is probably related to their role in the cells (19).

In mammals, oligopeptidases most likely participate in the intracellular catabolism of peptides. Studies on bacterial oligopeptidases suggested a role for OpdA of \textit{Salmonella enterica} serovar Typhimurium in the degradation of prolipoprotein signal peptides and in protein turnover (40). In lacticocci, casein hydrolysis by a cell envelope-associated protease results in the production of oligopeptides that, after internalization by a specific transport system, are hydrolyzed by peptidases into...
amino acids that are used for bacterial protein synthesis (39). These are then used for synthesis of bacterial proteins. Among the numerous peptidases identified, the chromosomal PepF oligopeptidase seems to be involved in protein turnover (14, 18). However, although PepF has been biochemically well characterized, its role in _L. lactis_ is still unclear.

### MATERIALS AND METHODS

#### Bacterial strains and growth conditions.

The _B. subtilis_ strains used in this study are listed in Table 1. Strains were grown in Schaeffer’s sporulation medium (6). Genetic transformations were carried out by the method of Anagnostopoulos and Spizizen (1). Transformants were selected on Schaeffer’s agar plates containing the appropriate antibiotic selection. Antibiotics were used at the following concentrations: chloramphenicol, 5 μg/ml; kanamycin, 2 μg/ml; and erythromycin, 25 μg/ml.

The _Escherichia coli_ DH5α strain was used for plasmid construction and propagation. Cells were grown in Luria-Bertani (LB) medium containing ampicillin (100 μg/ml) or kanamycin (20 μg/ml).

**Plasmid construction.** Plasmid pKK-I29 was isolated from a _B. subtilis_ chromosomal library constructed in the shuttle vector pHT315 (approximately 15 times) carries the _dacF-lacZ_ expressed in Miller units (16).

**Sporulation assay.** Sporulation assays were carried out in Schaeffer’s medium containing erythromycin at 25 μg/ml. Cells were grown for 24 or 48 h at 37°C, and then dilutions were plated before and after treatment with chloroform on Schaeffer’s agar plates containing erythromycin.

**β-Galactosidase assay.** Strains carrying _lacZ_ fusion constructs were grown in Schaeffer’s sporulation medium. Samples were taken at hourly intervals and assayed for β-galactosidase activity as previously described (7). Activity was expressed in Miller units (16).

### RESULTS

**Inhibition of sporulation by a multicopy _yjbG_ plasmid.** A _B. subtilis_ chromosomal library constructed in the replicative vector pHT315 (2, 41) was screened with the aim of identifying gene products whose overexpression would inhibit the activation of the phosphorelay or the activation of stage II gene expression. The screening procedure was designed to isolate bacteria impaired in σ^F-dependent transcription rather than sporulation. The plasmid library was transformed into a strain carrying the σ^F-dependent _dacF-lacZ_ fusion construct (strain JH11973) (Table 1) (43). Transformants were analyzed on 5-bromo-4-chloro-3-indolyl-β-D-galactopyranoside (X-Gal)-containing plates for their ability to drive transcription from the _dacF_ promoter as judged by the intensity of the blue color. Clones with a reduced transcription level were isolated, and the plasmid contained within was purified and used to transform strains carrying _spoIIA-lacZ_ (σ^H-dependent promoter) (42) and _sspE-lacZ_ (σ^E-dependent promoter) (36) (strains JH11624 and JH11974, respectively) (Table 1). All the plasmid clones that consistently reduced β-galactosidase expression from the _dacF_, _spoIIA_, and _sspE_ promoters were subjected to sequence analysis.

Plasmid pKK-I29 met these criteria and carried a 2,765-bp DNA fragment located at 105.1° on the _B. subtilis_ chromosome that spanned nucleotides 35603 to 38368 (GenBank accession number Z99110) (Fig. 1) (12). Two open reading frames were identified on this fragment by the genome sequencing project: the carboxy-terminal part of _yjbF_ and the entire _yjbG_, both followed by a putative transcription terminator. Strain JH642 carrying pKK-I29 was not only defective in _dacF_, _spoIIA_, and _sspE_ transcription but also sporulation defective. In order to determine whether the transcription and sporulation defects were due to overexpression of the truncated _yjbF_ or to the _yjbG_ gene, a kanamycin resistance gene was inserted in the _BalI_ and _MscI_ sites internal to _yjbG_. The resulting plasmid, pKK41, when transformed into the wild-type strain JH642, did not impair sporulation (data not shown), indicating that overexpression of _yjbG_ was likely to be responsible for the phenotypes of the strain carrying pKK-I29.

**_yjbG_ encodes the _PepF_ oligopeptidase.** The _yjbG_ gene encodes a protein with high sequence identity to the PepF1 and PepF2, respectively. Among the numerous peptidases identified, the chromosomal PepF oligopeptidase seems to be involved in protein turnover (14, 18). However, although PepF has been biochemically well characterized, its role in _L. lactis_ is still unclear.

**Materials and Methods.**

Bacterial strains and growth conditions. The _B. subtilis_ strains used in this study are listed in Table 1. Strains were grown in Schaeffer’s sporulation medium (6). Genetic transformations were carried out by the method of Anagnostopoulos and Spizizen (1). Transformants were selected on Schaeffer’s agar plates containing the appropriate antibiotic selection. Antibiotics were used at the following concentrations: chloramphenicol, 5 μg/ml; kanamycin, 2 μg/ml; and erythromycin, 25 μg/ml.

The _Escherichia coli_ DH5α strain was used for plasmid construction and propagation. Cells were grown in Luria-Bertani (LB) medium containing ampicillin (100 μg/ml) or kanamycin (20 μg/ml).

Plasmid construction. Plasmid pKK-I29 was isolated from a _B. subtilis_ chromosomal library constructed in the shuttle vector pHT315 (approximately 15 copies per cell) (2, 41). Plasmid pKK41 was derived from pKK-I29 by cloning a 1.4-kb kanamycin cassette gene (25) in the _BalI_ and _MscI_ sites of the _yjbG_ gene. Plasmid pKK9 was constructed in the transcriptional _lacZ_ fusion vector pDH32 (25). Plasmid pKK3 carrying the _dacF-lacZ_ fusion construct was obtained by cloning the _dacF_ promoter-containing 416-bp HindIII fragment (blunted by Klenow reaction) in the transcriptional fusion vector pDH32.

Sporulation assay. Sporulation assays were carried out in Schaeffer’s medium containing erythromycin at 25 μg/ml. Cells were grown for 24 or 48 h at 37°C, and then dilutions were plated before and after treatment with chloroform on Schaeffer’s agar plates containing erythromycin.

β-Galactosidase assay. Strains carrying _lacZ_ fusion constructs were grown in Schaeffer’s sporulation medium. Samples were taken at hourly intervals and assayed for β-galactosidase activity as previously described (7). Activity was expressed in Miller units (16).

### TABLE 1. _B. subtilis_ strains used in this study

<table>
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<tr>
<th>Strain</th>
<th>Relevant genotype</th>
<th>Source construction, or reference</th>
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<tbody>
<tr>
<td>JH642</td>
<td>trpC2 phe-1</td>
<td>Laboratory stock</td>
</tr>
<tr>
<td>JH646</td>
<td>trpC2 phe-1 spoA12</td>
<td>Laboratory stock</td>
</tr>
<tr>
<td>JH12545</td>
<td>trc2 phe-1 spoA12</td>
<td>Laboratory stock</td>
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<td>JH23009</td>
<td>trpC2 phe-1 pepC::aphA3</td>
<td>pKK41 (linear) → JH642</td>
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<td>JH11340</td>
<td>trpC2 phe-1 spoF::Y138</td>
<td>29</td>
</tr>
<tr>
<td>JH11435</td>
<td>trpC2 phe-1 spoF::Y318</td>
<td>29</td>
</tr>
<tr>
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<td>trpC2 phe-1 phrA::aphA3</td>
<td>M. Perego, unpublished</td>
</tr>
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<td>M. Perego, unpublished</td>
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<td>JH11975 (chr.) → JH646</td>
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<td>JH11975 (chr.) → JH2546</td>
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<td>pKK3 (linear) → JH642</td>
</tr>
<tr>
<td>JH11974</td>
<td>trpC2 phe-1 amyE::expE::2G::lacZ cat</td>
<td>pS1280 (linear) (36) → JH642</td>
</tr>
</tbody>
</table>

2. Arrow indicates construction by transformation.
an active Spo0A protein through an indirect mechanism, since no 0A box has been identified in the promoter region (data not shown). Spo0A regulation appears to be mediated, in part, by the AbrB transition state regulator protein (Fig. 2) (34).

Growth in nonsporulating conditions (Bacto-Antibiotic medium 3) resulted in an earlier induction of pepF transcription (T2) (data not shown), but the maximal level reached at T2 was comparable to the one obtained in Schaeffer’s medium.

Analysis of the nucleotide sequence of the putative pepF promoter did not reveal any distinctive motif indicative of a particular transcriptional regulatory mechanism.

Overexpression of pepF reverses the flow of phosphate in the phosphorelay. As described above, pKK-I29 was isolated as a multicopy plasmid that inhibited transcription from the σ^F-dependent dacF and sspE promoters. However, pKK-I29 also inhibited transcription from the spoIIA promoter (Fig 3A). Since the spoIIA operon encodes σ^F, inhibition of spoIIA expression could explain the inhibitory effect of pKK-I29 on dacF and sspE. It remained to be established whether the inhibitory effect caused by overexpression of pepF was exerted directly on the phosphorelay, thereby affecting the production of Spo0A~P required for spoIIA activation, or on an additional regulator(s) acting downstream of the phosphorelay.

In order to distinguish between these two possibilities, we analyzed the transcription pattern of abrB, a transition state regulator that, together with spoIIA, is commonly used as an indicator of the phosphorylation level of the Spo0A transcription factor. Spo0A~P is a positive activator of spoIIA transcription (38), while it acts as a repressor (or preventer) of abrB expression (35). By analyzing the changes occurring in the transcription from these two promoters in the presence of pKK-I29, we aimed to determine whether the inhibitory effect of pepF overexpression was exerted at the level of activation of the phosphorelay or at the level of activation of stage II genes.

As shown in Fig. 3A, overexpression of PepF lowered the...
level of transcription from the spoIIA promoter by approximately one third of the level in the wild-type strain, although the timing of activation was not significantly affected. This could be interpreted to mean that the level of Spo0A/H11011P in the cells was lower than in wild-type cells. However, repression of abrB transcription followed the wild-type pattern until the time of transition from exponential growth to sporulation (T0), indicating that the level of Spo0A/H11011P produced was sufficient to inhibit abrB expression. Nonetheless, immediately after T0, transcription of abrB progressively resumed (Fig. 3B).

This observation was reminiscent of the abrB transcription pattern resulting from a phrA mutation or from the rapA-spo0L892 mutation, which encodes a deregulated RapA protein unresponsive to inhibition by the PhrA peptide (29). In both cases, induction of rapA at the end of exponential phase by the ComP-ComA two-component system results in dephosphorylation of Spo0F-P. This, in turn, induces reverse phosphate flow in the phosphorelay and lowers the levels of phosphorylated Spo0A. The consequence of these events is derepression of the abrB promoter and resumption of its transcription. Notably, repression of abrB transcription and its resumption in the pepF-expressing strain, as well as sporulation efficiency, were not as drastically affected as in the phrA null mutant or in the rapA-spo0L892 strain. Thus, overexpression of PepF appeared to act at the level of the phosphorelay, most likely through modulation of the negative role played by phosphatases.

Overexpression of PepF results in intracellular processing of PhrA. The PepF1 oligopeptidase from L. lactis was shown to hydrolyze peptides containing between 7 and 17 amino acids with a rather wide sequence specificity (18). Furthermore, it was noted that PepF1 often cleaved Phe-X bonds (with X being a hydrophilic or basic residue). Although it appeared that the size of the substrate peptides may be an important factor for their hydrolysis by PepF1, it was tempting for us to speculate that the sporulation deficiency caused by overexpression of PepF in B. subtilis could be the result of hydrolysis of the PhrA peptide. However, since the pepF-overexpressing strain has a less dramatic sporulation phenotype than a phrA null mutant (22% of sporulation efficiency versus approximately 6%), one would have to assume that not all of the PhrA molecules are targeted by PepF but some are actually able to proceed through the regular maturation steps and result in an active pentapeptide form.

In any event, intracellular processing of some PhrA molecules would impair its maturation through the export-import

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**Fig. 3.** Effect of PepF overexpression on spoIIA and abrB transcription. Samples for β-galactosidase activity were taken for the spoIIA-lacZ fusion (A) and the abrB-lacZ fusion (B). Cells were grown in Schaeffer’s sporulation medium containing erythromycin at 25 μg/ml. Symbols: ■, JH16124/pHT315; ○, JH16124/pKK-I29; ▲, JH12604/pHT315; Δ, JH12604/pKK-I29.
control circuit and thus formation of active PhrA pentapeptide inhibitor at the wild-type concentration needed to fully inhibit RapA phosphatase. This would have the consequence of (i) leaving the RapA phosphatase deregulated, (ii) inhibiting sporulation, and (iii) derepressing abrB through regulation of ComA-dependent genes (13, 33). A deletion of the yusX gene (chromosomal coordinates 3379766 to 3381265) encodes a protein with similarity to PepF. YusX is only 500 amino acids in length, with a molecular mass of 57,565 Da. Its first methionine aligns to the sequence of PepF starting at residue 103, with 19% identical residues and 19.6% conserved substitutions. Analysis of this chromosomal region revealed that yusX is immediately preceded by an open reading frame (ORF),

<table>
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<th>No. of viable cells</th>
<th>No. of spores</th>
<th>% Sporulation</th>
</tr>
</thead>
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<tr>
<td>JH642/pKK-I29</td>
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<tr>
<td>JH11430/pHT315</td>
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<td>JH11430/pKK-I29</td>
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<td>6.2 × 10^6</td>
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<tr>
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<td>4.5</td>
</tr>
</tbody>
</table>

*Representative of several independent experiments carried out at 24 or 48 h of growth. Cells were grown in Schaeffer’s sporulation medium supplemented with erythromycin at 25 μg/ml.

### TABLE 2. Efficiency of sporulation of strains carrying the multicopy pepF plasmid pKK-I29 or the control vector pHT315

Overexpression of PepF affects the activity of the PhrC competence factor. We investigated whether the *B. subtilis* PepF was processing additional peptides of the Phr family when overexpressed. We observed that the sporulation deficiency of a *pheA* null mutant strain (JH11842) was partially suppressed by the presence of plasmid pKK-I29 compared to the vector control pHT315. A similar level of suppression was observed when the *pheA* null mutation was combined with a deletion of the *pheC* gene encoding the PhrC peptide (CSF) for competence and sporulation (13) (20% of sporulation efficiency compared to 6% of the *pheA* mutant alone) (unpublished data). The PhrC pentapeptide (ERGMT) is known to affect the transcription of ComA-dependent genes (13, 33). A deletion of *pheC* decreases the level of transcription from the ComA-dependent *rapA* promoter, and this results in increased sporulation efficiency (20) (unpublished data).

We reasoned that if PepF were processing PhrC, transcription of *rapA* would decrease in a strain carrying the multicopy pKK-I29 plasmid compared to a strain carrying the vector alone. In order to overcome the effect inherent in the sporulation-deficient phenotype of a pKK-I29-carrying strain on ComA-dependent transcription, the experiment was carried out on a strain with a deletion of the *pheA* gene and thus already sporulation deficient. β-Galactosidase assays (Fig. 4) carried out on a strain containing the *rapA-lacZ* fusion construct showed that, in a *pheA* mutant strain, transcription of *rapA* was negatively affected by overexpression of PepF. This indicated that some factor(s) required for full activation of this promoter was impaired. When transcription of *rapA* was analyzed in a *pheA* pheC double mutant strain, the negative effect caused by pKK-I29 was abolished. These results suggested that the product of *pheC* as well as the product of *pheA* may be a target of the PepF peptidase overproduction at some stage of its maturation/activation pathway.

### PepF Oligopeptidase of *Bacillus subtilis*

Figure 4. Effect of PepF overexpression on ComA-dependent gene transcription. Samples for β-galactosidase activity were taken for the *rapA-lacZ* fusion construct. Strains were grown in Schaeffer’s sporulation medium supplemented with erythromycin at 25 μg/ml. Symbols: •, JH11909/pHT315; ▲, JH11909/pKK-I29; ○, JH11908/pHT315; △, JH11908/pKK-I29.
yusY (chromosomal coordinates 3381265 to 3381558), with similarity to the amino-terminal domain of oligopeptidases. Additionally, yusY was preceded by a putative promoter region containing typical −10 and −35 consensus sequences for ρ^− containing RNA polymerase, and the ATG start codon was preceded by a ribosome-binding site (GGG GT). In contrast, yusX did not appear to be preceded by a canonical ribosome-binding site and/or promoter sequence. This suggested that yusY and yusX may actually be a single ORF, as it is in B. halodurans (37), were it not for a frameshift due to a sequence error.

Resequencing of this region from strain JH642, however, did not reveal any error, indicating that, at least in the laboratory strains, this putative yusXY gene product is truncated and presumably inactive. The putative YusXY protein (598 amino acids) is most likely a metalloprotease, given the presence of the zinc-binding motif HELGH in its sequence. The absence of a potential signal peptide sequence suggests that YusXY, like PepF, is a cytoplasmic protein. The region containing the yusX and yusY genes and upstream promoter was amplified by PCR, cloned on the multicopy vector pHT315, and transformed into the wild-type strain JH642. This strain did not show any sporulation-deficient phenotype on Schaeffer’s sporulation medium plates, and it produced an amount of CHCl_3-resistant spores in liquid culture comparable to that produced by the control strain carrying the vector alone (data not shown). Since no further investigation on yusXY was carried out, its function in the cell, if any, remains unknown.

**DISCUSSION**

We report the identification of a B. subtilis gene, yjbG, encoding a protein homologous to the PepF1 and PepF2 oligopeptidases of L. lactis (18, 21). The B. subtilis PepF protein shows 45% and 46% identical residues with PepF1 and PepF2, respectively, suggesting a functional conservation. Here we propose to rename yjbG as pepF.

The role of oligopeptidases in bacterial growth is still relatively unclear. It has been proposed that the OpdA oligopeptidase of S. enterica serovar Typhimurium is involved in protein turnover during carbon deficiency and also carries out degradation of signal peptides (40). Similarly, PepF1 and PepF2 of Lactococcus could be involved in protein turnover. It was reported that a mutant devoid of PepF activity was viable in rich medium but grew poorly in minimal medium (21). Since starvation for required nutrients leads to an elevated rate of protein degradation (15), the beneficial effect of both PepF activities present in the cell grown in minimal medium suggested an important role played by oligopeptidases. Additionally, the amplification of the level of expression of the plasmid-encoded PepF1 may have provided a selective advantage. A protein showing 30% homology to PepF1 was identified in Mycoplasma genitalium, whose small genome is thought to contain a minimal set of genes essential for life, given the small size of its chromosome. A global transposon mutagenesis study suggested that in this organism pepF may be an essential gene (9).

Although the role of PepF in B. subtilis physiology is still unknown, it is apparent that amplification of its cellular level affects sporulation and competence development in a manner consistent with a degradation activity toward Phr peptides. The fact that the sporulation defect caused by overproduction of PepF can be overcome by mutations that relieve the system from the inhibitory role of PhrA (deletion of rapA or spo0F mutations that render the response regulator insensitive to Rap-dependent dephosphorylation) strongly points to PhrA as a target for PepF-dependent hydrolysis. Support for a role in processing of Phr peptides in PepF overexpression conditions is provided by the observation that a ComA-dependent gene such as rapA was affected by overproduction of PepF, and this effect was overcome by a deletion of phrC. The PhrC peptide is known to affect ComA-dependent gene transcription, mainly by acting through the RapC aspartyl phosphate phosphatase (13). Although the biochemical mechanism of this regulatory control is still undefined, the in vivo effect of changing the concentration of PhrC in the cells is detectable in minimal medium as well as in growth conditions that promote sporulation (Fig. 4).

Our results also indicate that a PhrA peptide as small as the active pentapeptide can be targeted for proteolysis by PepF. Although all the oligopeptidases of the M3 family are believed to hydrolyze peptides containing not fewer than 5 amino acids and not more than 20, it is also believed that, within the family, great differentiation exists in substrate specificity (23, 24). The B. subtilis PepF, then, falls into this group, although its specificity may be affected by the condition of protein overproduction obtained with the multicopy plasmid.

This raises the question of whether PepF-dependent hydrolysis of Phr peptides may have a physiological role. The pepF gene is transcribed at a low level during exponential phase and is induced twofold immediately after the transition to stationary phase. This induction is dependent on the sporulation transcription factor Spo0A. Whatever PepF’s functions may be, it could contribute to the onset of the sporulation process. It seems unlikely, however, that processing of PhrA is a required function of PepF. In fact, inactivation of PhrA would most likely result in a delay in, if not inhibition of, sporulation initiation because of the resulting uncontrolled activity of the RapA phosphatase. In the case of PhrC, on the contrary, one may consider that inactivation of this peptide would result in deregulation of RapC, which, in turn, would inhibit competence development, therefore allowing sporulation to initiate.

We are presently unable to assign a function to the product of pepF in B. subtilis because inactivation of the chromosomal gene did not cause any detectable phenotype in sporulation growth conditions. Furthermore, deletion of pepF did not affect cell growth in rich or minimal medium (data not shown). Therefore, contrary to what has been observed in Lactococcus and M. genitalium, the PepF oligopeptidase is not necessarily involved in protein turnover as a survival response in conditions of nutrient starvation. It is also possible that redundant activities exist that can compensate for the absence of PepF. In B. subtilis, this alternative activity does not seem to be provided by the oligopeptidase YusXY, as the corresponding ORF is disrupted. However, in other bacilli such as B. halodurans, the presence of an intact YusXY protein suggests that it may have overlapping functions with PepF.

It is possible that PepF functions in degradation of signal peptides, as suggested for the OpdA oligopeptidase (40). Two signal peptide peptidases, SppA and TepA, have been identified in B. subtilis, and they are specifically required for the
degradation of preproteins or signal peptides that are inhibitory to protein translocation (3). We cannot rule out the possibility that PepF contributes to this activity, since it is not known whether a double sppA tepA mutant carries any residual degradation activity that could be accounted for by the presence of the oligopeptidase.

Analysis of the nucleotide sequence of the pepF promoter did not identify any motif suggestive of a particular transcriptional regulatory mechanism. Transcription of pepF in sporulation conditions is relatively low; however, a twofold increase occurs in the postexponential phase as mentioned earlier, with a sharp decline after approximately 3 h in the stationary phase. A similar pattern of transcription has also been observed for the sppA gene, and this was interpreted as being consistent with the hypothesis that SppA has signal peptide peptidase activity required to prevent the accumulation of cleaved signal peptides in conditions of high-level synthesis of secretory proteins. However, while SppA is a membrane protein, PepF is a cytosolic protein, and therefore, if this were its function, it may be targeting it quite distinct proteins.

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