

## Differential Expression of Two Paralogous Genes of *Bacillus subtilis* Encoding Single-Stranded DNA Binding Protein

Cordula Lindner,<sup>†‡</sup> Reindert Nijland,<sup>†</sup> Mariska van Hartskamp,<sup>§</sup> Sierd Bron,  
Leendert W. Hamoen,<sup>¶</sup> and Oscar P. Kuipers\*

Department of Genetics, Groningen Biomolecular Sciences and Biotechnology Institute, NL-9751 NN Haren,  
The Netherlands

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**The *Bacillus subtilis* genome comprises two paralogous single-stranded DNA binding protein (SSB) genes, *ssb* and *ywpH*, which show distinct expression patterns. The main *ssb* gene is strongly expressed during exponential growth and is coregulated with genes encoding the ribosomal proteins S6 and S18. The gene organization *rpsF-ssb-rpsR* as observed in *B. subtilis* is found in many gram-positive as well as some gram-negative bacteria, but not in *Escherichia coli*. The *ssb* gene is essential for cell viability, and like other SSBs its expression is elevated during SOS response. In contrast, the paralogous *ywpH* gene is transcribed from its own promoter at the onset of stationary phase in minimal medium only. Its expression is ComK dependent and its gene product is required for optimal natural transformation.**

Single-stranded DNA binding proteins (SSBs) in bacteria play crucial roles in DNA replication, repair, and recombination processes. The function of SSB in these processes, its biochemical properties, and its interaction with other proteins in the cell have been studied extensively in *Escherichia coli* and several bacteriophages (17, 26). Relatively little is known about the regulation of SSB expression.

In *Escherichia coli* the *ssb* gene is preceded by three promoters, one of them being inducible by DNA damage (3, 4). The DNA damage inducibility is due to the presence of a LexA binding site in the upstreammost promoter. Interestingly, this SOS-box is shared with the divergently transcribed *uvrA* gene, coding for the A subunit of the exonuclease ABC, which is involved in DNA repair (3). The same organization was found for the *uvrA* and *ssb* genes in *Sinorhizobium meliloti* (24). Although this gene organization is also identical in *Proteus mirabilis* and *Serratia marcescens*, the *ssb* genes of these bacteria are not inducible by DNA damage (7). It has been suggested that *E. coli* SSB negatively autoregulates its own translation, because it is capable of binding to its own mRNA, in this way inhibiting translation (21).

Two paralogous genes coding for SSB were found in the *Bacillus subtilis* genome, *ssb* and *ywpH*. The deduced amino acid sequences of SSB and YwpH show 80% similarity and 63% identity. Notably, YwpH is lacking 66 amino acid residues of the C terminus of SSB. Although the amino acid sequences of bacterial SSBs are highly conserved within the first two

thirds of the protein containing the DNA-binding domains, they diverge substantially in the C-terminal third region (7). The C-terminal region of *E. coli* SSB is not required for DNA binding in vitro, but is essential for its in vivo function (6, 29). In contrast to *E. coli*, neither of the *B. subtilis* *ssb* genes is organized adjacent to *uvrA* as the *ssb* gene is in *E. coli*. The first one, *ssb*, maps at 358.6° of the *B. subtilis* genome and is flanked by the *rpsF* and *rpsR* genes, coding for the ribosomal proteins S6 and S18, respectively (Fig. 1A). A rho-independent transcriptional terminator is situated downstream of the *rpsR* gene, and possibly *rpsF*, *ssb*, and *rpsR* belong to one operon. The second *ssb*-like gene, *ywpH*, maps at 319.4° and is flanked by a gene of unknown function (*ywpG*) and the *glcR* gene, coding for a regulator involved in carbon catabolite repression (23) (Fig. 1B). Between these genes, no obvious terminator structure could be identified.

In this paper we studied the transcriptional regulation of the two *ssb*-like genes in *B. subtilis* and address the question of why there are two SSBs in this organism.

### MATERIALS AND METHODS

**Bacterial strains, medium, and growth conditions.** Table 1 lists the bacterial strains and plasmids used in this study. Bacteria were grown at 37°C under vigorous agitation in rich medium (TY [1% tryptone, 0.5% yeast extract, 1.0% NaCl] or BFA [16] when appropriate) or minimal medium (22). For the selection of transformants, appropriate antibiotics were added to the growth media at the following concentrations: for *B. subtilis*, 5 µg of chloramphenicol per ml, 10 µg of kanamycin per ml, and 100 µg of spectinomycin per ml; for *E. coli*, 100 µg of ampicillin per ml and 150 µg of spectinomycin per ml. To visualize  $\alpha$ -amylase activity, TY plates were supplemented with 1% starch, and to visualize LacZ activity plates were supplemented with 0.004% 5-bromo-4-chloro-3-indolyl- $\beta$ -D-galactopyranoside (X-Gal).

**Strain constructions and transformation.** Cloning and transformation were performed according to established techniques (5), (20) and suppliers' manuals. The nucleotide sequences of the primers used for PCR are listed in Table 2. Enzymes were from Roche Molecular Biochemicals (Mannheim, Germany).

Upstream regions of the *ssb* and *ywpH* genes were amplified by PCR with *Pwo* DNA polymerase and chromosomal DNA of *B. subtilis* 168 as the template. The following fragments were amplified: fragment S1 (primers *ssb*-1 and *ssb*-2; from 295 bp upstream to 11 bp downstream of the start codon of *ssb*), S2 (primers *rps*-1, *ssb*-2; from 602 bp upstream to 11 bp downstream of the start codon of

\* Corresponding author. Mailing address: Department of Genetics, Groningen Biomolecular Sciences and Biotechnology Institute, Kerklaan 30, NL-9751 NN Haren, The Netherlands. Phone: 31 50 3632093. Fax: 31 50 3632348. E-mail: o.p.kuipers@biol.rug.nl.

<sup>†</sup> C.L. and R.N. contributed equally to this article.

<sup>‡</sup> Present address: FCDF Corporate Research, 7418 BA Deventer, The Netherlands.

<sup>§</sup> Present address: PURAC Biochem, 4200 AA Gorinchem, The Netherlands.

<sup>¶</sup> Present address: Sir William Dunn School of Pathology, University of Oxford, Oxford OX1 3RE, United Kingdom.

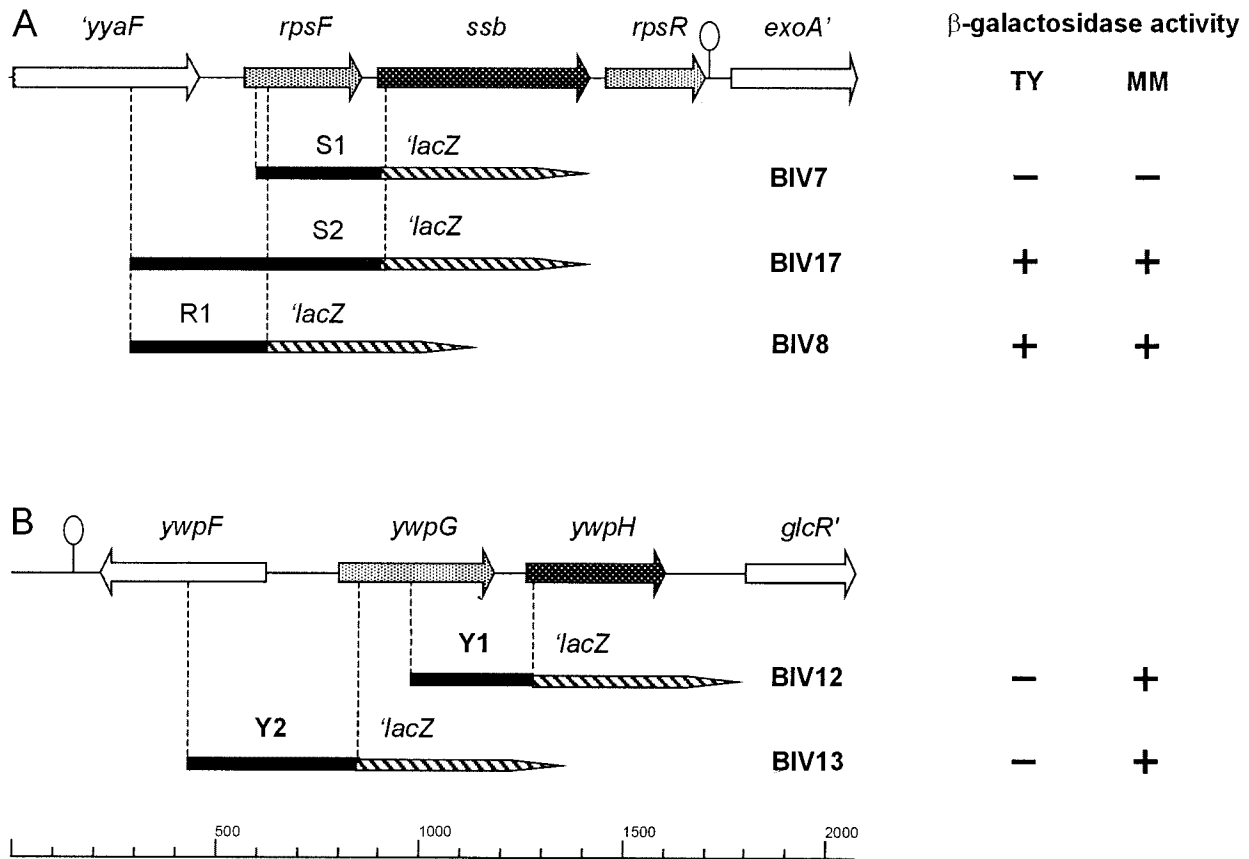


FIG. 1. Gene organization of the *ssb* operon (A) and the *ywpH* gene (B) in *B. subtilis* 168, with schematic representation of the constructed *lacZ* fusions. Black lines in bold type represent the PCR-amplified DNA fragments fused to the promoterless *lacZ* gene (*lacZ* gene not drawn to scale). The presence or absence of β-galactosidase activity in a certain construct after growth on rich (TY) or minimal (MM) medium is indicated on the right site by + and -, respectively.

*ssb*), R1 (primers *rps*-1, *rps*-2; from 274 bp upstream to 50 bp downstream of the start codon of *rpsF*), Y1 (primers *ywpH*-1, *ywpH*-2; from 282 bp upstream to 10 bp downstream of the start codon of *ywpH*), and Y2 (primers *ywpG*-1, *ywpG*-2; from 369 bp upstream to 50 bp downstream of the start codon of *ywpG*). These PCR fragments were cloned into the *Sma*I-digested promoter-screening vector

pBTK2 (15). The resulting plasmids carrying the insert in the correct orientation were linearized and transformed into *B. subtilis* 168, selecting for kanamycin-resistant transformants. The transformants were screened for an amylase-deficient phenotype to confirm that the construct had integrated in the *amyE* locus.

The *ywpH* deletion mutant was constructed as follows. A 1.493-kb DNA

TABLE 1. *B. subtilis* strains and plasmids used in this study

Strain or plasmid	Relevant characteristics	Source or reference
<b>Strains</b>		
168	<i>trpC2</i>	1
BIV7	<i>trpC2 amyE::(ssb'-lacZ kan) Km<sup>r</sup></i>	This study
BIV8	<i>trpC2 amyE::(rpsF'-lacZ kan) Km<sup>r</sup></i>	This study
BIV12	<i>trpC2 amyE::(ywpH'-lacZ kan) Km<sup>r</sup></i>	This study
BIV13	<i>trpC2 amyE::(ywpG'-lacZ kan) Km<sup>r</sup></i>	This study
BIV17	<i>trpC2 amyE::(rpsF ssb'-lacZ kan) Km<sup>r</sup></i>	This study
BIV24	<i>trpC2 amyE::(ywpH-lacZ kan) comK::spec Km<sup>r</sup> Sp<sup>r</sup></i>	This study
BIV27	<i>trpC2 ywpH::cat Cm<sup>r</sup></i>	This study
BV2004	<i>trpC2 his met tyr-1 ade nic ura comK::spec Sp<sup>r</sup></i>	12
<b>Plasmids</b>		
pBTK2	<i>E. coli</i> Ap <sup>r</sup> ; <i>B. subtilis</i> Km <sup>r</sup> , <i>amyE</i> back and front, promoterless <i>lacZ</i> gene for reporter gene expression studies	15
pIC408	Sp <sup>r</sup> , <i>ori</i> pUC, multiple cloning site of pBluescript II SK(+), suicide plasmid for <i>B. subtilis</i> to construct "knockout" mutants	M. Steinmetz, unpublished
pBluescript SK-pUC7c	Ap <sup>r</sup> , T4 and T7 promoter/RNA polymerase site for synthesizing RNA probes pUC21 derivative bearing chloramphenicol resistance cassette	Stratagene 11

TABLE 2. Primers used in this study

Primer	Description	Sequence (5' to 3') <sup>a</sup>
rpsF-3	Forward primer to amplify the <i>rpsF</i> ( <i>ssb</i> ) promoter region without RBS	<u>GGAATTCCTGCAGGTGACTTTGAGCGGGGCTTC</u>
rpsF-4	Reverse primer to amplify the <i>rpsF</i> ( <i>ssb</i> ) promoter region without RBS	GGCCTCGAGGGCCCATAAATGGGCAAGGAGC
rpsF-5	Reverse primer to amplify the <i>rpsF</i> ( <i>ssb</i> ) promoter region with RBS	CGTACTTTCTCATATGTTTGACC
ssb-1	Forward primer to amplify the <i>ssb</i> promoter region	GCGAAGCTTCCAAACATTGACGAAGAGTCT
ssb-2	Reverse primer to amplify the <i>ssb</i> promoter region	GCTGGATCCTCGGTTAAGCATAAGAAAGACC
rps-1	Forward primer to amplify the <i>rpsF</i> promoter region	<u>GCGAAGCTTGTGACTTTGAGCGGGGCTT</u>
rps-2	Reverse primer to amplify the <i>rpsF</i> promoter region	GCTGGATCCATCTTCGTCAATGTTTGGGCG
yyaE-1	Forward primer to amplify the <i>yyaF</i> promoter region	GCGAAGCTTGAAGCATGAAGGGCAAGGTG
yyaE-2	Reverse primer to amplify the <i>yyaF</i> promoter region	GCTGGATCCTCCGACGTTCCGGCAAACCAA
ywpG-1	Forward primer to amplify the <i>ywpG</i> promoter region	GCGAAGCTTGGCTTCAGATTGGCTGTTTTG
ywpG-2	Reverse primer to amplify the <i>ywpG</i> promoter region	GCTGGATCCTCAGAAGGAACGCCGTCAATA
ywpH-1	Forward primer to amplify the <i>ywpH</i> promoter region	CCCAAGCTTTCAAGCTGTCAATGCCG
ywpH-2	Reverse primer to amplify the <i>ywpH</i> promoter region	CGCGGATCCGATTGAACATGCGATTCC
X-ywpH-3	Forward primer to amplify the <i>ywpGH-glcR</i> region	GCCGCTCGAGGACTATGGATTACGGAGAGATGTGG
H-ywpH-4	Reverse primer to amplify the <i>ywpGH-glcR</i> region	GCCAAGCTTGCTCCTTTTCCAGCTTGCTCC

<sup>a</sup> The hexameric restriction sites are underlined.

fragment containing the *ywpH* gene flanked by 486 bp of upstream sequence and 665 bp of downstream sequence was amplified by PCR with primers X-ywpH-3 and H-ywpH-4 and chromosomal DNA of *B. subtilis* 168 as a template. The amplicon was cloned into *Pvu*II-digested plasmid pIC408, a pUC derivative carrying a spectinomycin resistance gene (M. Steinmetz, unpublished data). Subsequently, a 150-bp internal fragment of *ywpH* was deleted in the resulting plasmid by *Pvu*II and *Sma*I digestion and replaced with the 1.34-kb *Pvu*II fragment of pUC7c (11) containing the chloramphenicol resistance marker. The resulting plasmid was transformed into *B. subtilis* 168 and BIV12. Transformants were selected for chloramphenicol resistance and subsequently screened for spectinomycin sensitivity, indicating the successful disruption of *ywpH* due to a double-crossover event.

The *comK* disruption mutant was constructed by transforming *B. subtilis* BIV12 carrying the Y1-*lacZ* fusion with chromosomal DNA of strain BV2004 carrying a spectinomycin cassette integrated into the *comK* gene (12). The resulting spectinomycin- and chloramphenicol-resistant strain was designated BIV24 and used for transcription studies.

**$\beta$ -Galactosidase activity assay.** To assay  $\beta$ -galactosidase activities, overnight cultures were diluted in fresh medium and samples were taken at different intervals for optical density readings at 600 nm (OD<sub>600</sub>) and  $\beta$ -galactosidase activity determinations. The  $\beta$ -galactosidase assay and the calculation of  $\beta$ -galactosidase units (Miller units) were performed as described by Miller (18).

**Northern blot analysis.** Total RNA of *B. subtilis* was isolated from cultures grown in TY, BFA, or MM. Cells were harvested at hourly intervals, from 3 h before until 3 h after transition point (T), and RNA was extracted as previously described (12); 1  $\mu$ g of total RNA was separated by formaldehyde-agarose gel electrophoresis and blotted onto a nylon membrane. This membrane was hybridized with digoxigenin-labeled probes detecting transcripts containing *yyaF*, *rpsF*, *ssb*, or *ywpH*, respectively. Probes were constructed by inserting internal fragments of the coding regions of *yyaF*, *rpsF*, *ssb*, or *ywpH* in the multiple cloning site of the pBluescriptSK(-) plasmid. Subsequently digoxigenin-labeled antisense RNA probes were transcribed in vitro with the T7 or T3 RNA polymerase site present in this plasmid. In vitro RNA labeling, hybridization, and signal detection were carried out according to the manufacturer's instructions (DIG Northern starter kit; Roche Diagnostics, Mannheim, Germany).

**Induction of DNA damage.** Cells of the strains carrying either the S1- or the R1-*lacZ* fusion were grown in TY and cells carrying the Y1-*lacZ* fusion were grown in MM. After they reached an OD<sub>600</sub> of about 0.1, the cultures were divided and mitomycin C was added to one portion of the culture at a final concentration of 100 ng/ml and the expression of *ssb* and *ywpH* was monitored.

**Assessment of competence.** To test the involvement of YwpH in natural competence a deletion was introduced into the *ywpH* gene of *B. subtilis* and transformability assays were performed as described previously (5).

## RESULTS AND DISCUSSION

**Transcription analysis of the *ssb* and *ywpH* genes.** To study the expression of the *ssb* and *ywpH* genes, transcriptional fusions of the potential promoter-containing fragments with a promoterless *lacZ* were constructed and integrated into the *B. subtilis* chromosome at the site of *amyE*.

Strains BIV7 (S1), BIV17 (S2), BIV8 (R1), BIV12 (Y1), and BIV13 (Y2) containing the different *lacZ* fusions, schematically depicted in Fig. 1, were screened on rich (TY) and minimal medium (MM) agar plates containing X-Gal for blue or white phenotypes. This revealed promoter activity only for the constructs in strains BIV17, BIV8, BIV12, and BIV13, but not in BIV7. In BIV12 and BIV13, promoter activity was detected only in MM, indicating a medium-dependent expression of the genes *ywpG* and *ywpH* (Fig. 1).

No promoter activity could be detected when the 295 bp immediately upstream of the *ssb* start codon (S1) were used to drive *lacZ* expression (Fig. 1). However, strong promoter activity was detected with the S2 fragment containing the complete *rpsF* gene and 274 bp upstream of the *rpsF* start codon. Apparently, *ssb* and *rpsF* are part of one operon, which presumably also includes *rpsR*. This is confirmed by the fact that promoter activity was also found from the smaller R1 fragment comprising the 274 bp upstream of the *rpsF* start codon only. In contrast, *ywpH* was found to be transcribed from a promoter directly upstream of the gene.

In order to study the expression pattern of *ssb* and *ywpH* in more detail, strains containing the S1-, R1-, and Y1-*lacZ* fusions (BIV7, BIV8, and BIV12, respectively) were grown in TY and MM and  $\beta$ -galactosidase activity was examined. No expression could be detected from the S1 fragment under the conditions employed (data not shown), but the *ssb* operon appeared to be strongly transcribed from the *rpsF* promoter in both rich and minimal media. The highest values (between 200 and 300 Miller units per OD) were reached during exponential growth (Fig. 2A and B). After the transition point (T) between

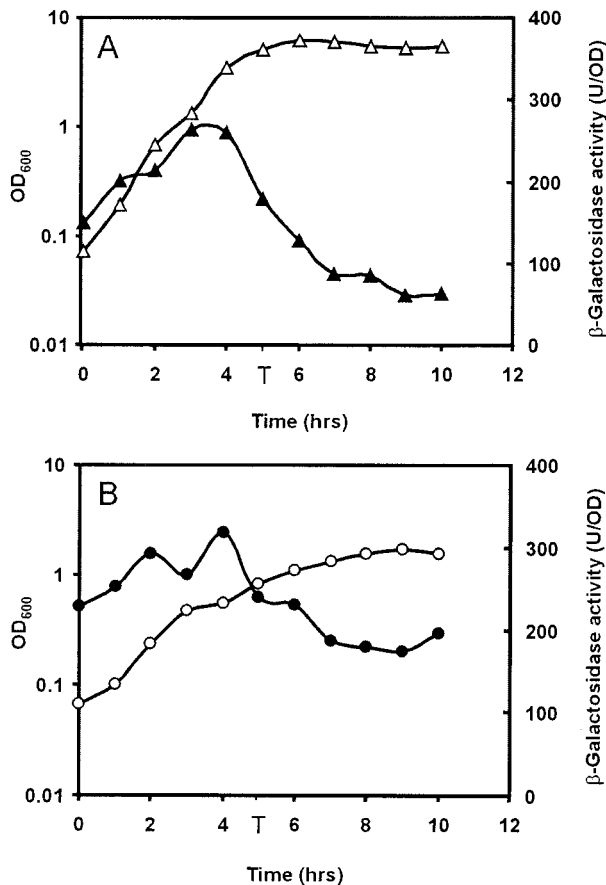


FIG. 2. Expression of the *ssb* operon in *B. subtilis*. Growth (open symbols) in rich (triangles) or minimal (circles) medium and expression of the transcriptional *lacZ* fusions (solid symbols) with the *rpsF* gene, the first gene of the *ssb* operon, reflected as β-galactosidase activity per OD. T indicates the time point at which transition from logarithmic to stationary growth takes place.

logarithmic growth and stationary growth, transcription from the *rpsF* promoter decreased. There is still transcription in the stationary phase at a two- to fourfold lower level. These results are in agreement with the higher need for SSB protein for DNA replication in fast-growing and thus frequently dividing cells. In contrast, no expression of the *ywpH* gene could be detected in cells of *B. subtilis* BIV12 grown in TY and only low expression was observed in exponentially growing cells in MM. However, expression of *ywpH* was strongly induced when cells entered the stationary phase and reached its highest level after the transition point (Fig. 3).

**Northern blot analysis.** For a more detailed picture of the transcription of *ssb* and *ywpH*, Northern blots with probes for *yyaF* (upstream of *rpsF*), *rpsF*, *ssb*, and *ywpH* were performed. Upstream of *ssb* two promoters are present, a promoter upstream of *rpsF* and a promoter upstream of *yyaF*. Downstream of *rpsR* a terminator structure is present. Transcription from these two promoters would result in two mRNA fragments. A transcript containing only *rpsF-ssb-rpsR* of 1.2 kb and a transcript also containing *yyaF* of 2.4 kb (Fig. 1A). Figure 4A shows fragment sizes of 2.4 and 1.3 kb with the *rpsF* probe, which correspond to the expected transcripts. With the *ssb* probe, a

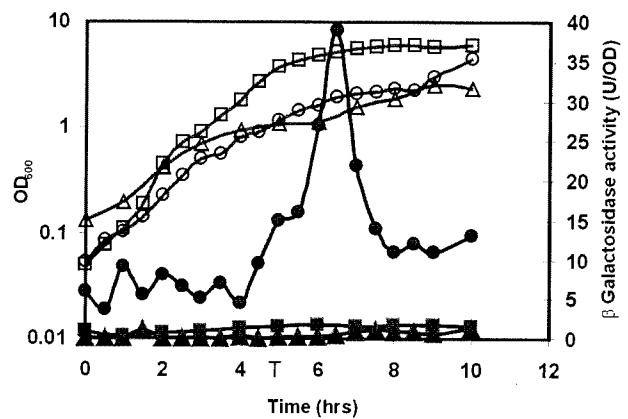


FIG. 3. Expression of the *ywpH* gene in *B. subtilis* and effect of a *comK* mutation. Growth (open symbols) in rich (squares) or minimal (circles) medium and expression of the transcriptional *lacZ* fusions (solid symbols) of the *ywpH* gene in strain BIV12 reflected as β-galactosidase activity per OD. The effect of a *comK* mutation on *ywpH* expression is also shown. Growth in minimal medium (open triangles) and expression of the transcriptional *ywpH-lacZ* fusion reflected as β-galactosidase activity per OD (solid triangles) in the *comK* mutant BIV24. T indicates the time point at which transition from logarithmic to stationary growth takes place.

smaller fragment of about 0.9 kb is also visible. Since no promoter activity was detected directly upstream of *ssb*, this fragment is likely to be caused by selective cleavage or degradation of the mRNA leading to the removal of *rpsF* from the *ssb-rpsR* fragment. The size of such a fragment would be 0.85 kb, which corresponds to the size observed.

In MM *ssb* and *rpsF* are transcribed highly during logarithmic growth from their own promoter, and after the transition point they are also transcribed from the *yyaF* promoter. The results from both the β-galactosidase assay and the Northern blot analyses suggest that *ssb* is cotranscribed and coregulated with genes coding for ribosomal proteins, thereby coupling the regulation of protein synthesis to DNA metabolism.

In rich medium *yyaF* is transcribed during log phase and its transcription stops at the onset of stationary phase (Fig. 4A). However, in minimal medium *yyaF* is highly transcribed after the transition point (Fig. 4B). The RNA fragment that hybridizes with the *yyaF* probe corresponds to the upper band visible in both the *rpsF* and *ssb* blots. This indicates that transcription from the *yyaF* promoter continues into the *rpsF-ssb-rpsR* operon, causing additional transcription of this operon from the *yyaF* promoter. In MM the transcription of *rpsF*, *ssb*, and *rpsR* is therefore boosted from the *yyaF* promoter, which itself is shown to be ComK dependent by means of DNA array analysis (2, 12, 19). Two of these studies also showed the transcription of *rpsF* and *ssb* to be ComK dependent. Our results show that it is likely that this observation is caused by readthrough from the *yyaF* promoter rather than direct regulation of the *rpsF* promoter by ComK. The apparent transcription of *yyaF* in rich medium during late logarithmic phase indicates multiple modes of transcription regulation of this gene.

The transcription of *ywpH* in TY is virtually absent, but two clear mRNA fragments are present when the cells were grown in MM (Fig. 4C). The fragments observed have sizes of 2.4 and

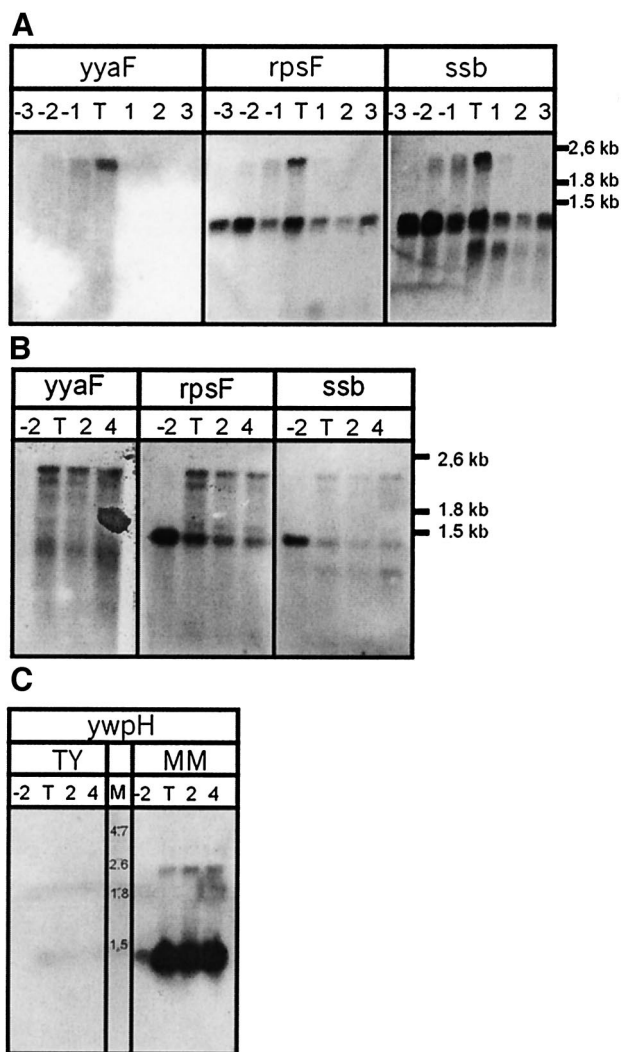


FIG. 4. Transcription of the *ssb* operon and *ywpH* in *B. subtilis*. (A) RNA samples were taken from wild-type cells growing in rich medium (BFA) at hourly intervals, from 3 h before until 3 h after transition point (T). The blots were hybridized with RNA probes detecting transcripts containing *yyaF*, *ssb*, or *rpsF*. (B) RNA samples were taken from wild-type cells growing in MM at -2 h, transition point (T), +2 h, and +4 h. The blots were hybridized with RNA probes detecting transcripts containing *yyaF*, *ssb*, or *rpsF*. (C) RNA samples from wild-type cells growing in TY or MM were taken at -2 h, transition point, +2 h, and +4 h. The blots were hybridized with RNA probes detecting transcripts containing *ywpH*.

1.4 kb. The 1.4-kb band corresponds to a transcript containing *ywpH* and *glcR*. The longer fragment observed at time points 2 and 4 of 2.4 kb is likely to be a result of readthrough into the downstream gene *ywpJ* due to an enhanced transcription rate. During logarithmic growth a small amount of *ywpH* containing mRNA is detected, and when the cells reach stationary growth the transcription of *ywpH* increases.

In conclusion, the two *ssb*-like genes obviously show opposite expression patterns, one being expressed to the highest level during exponential growth and the other one being expressed during the stationary phase in MM, indicating a distinct function of their gene products. These results should be

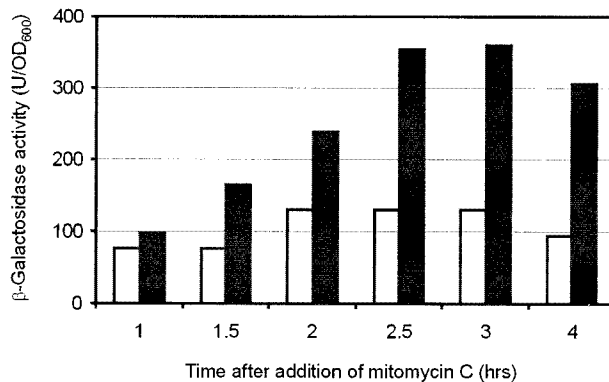


FIG. 5. Effect of DNA damage on expression of the *ssb* operon. Expression of the transcriptional *rpsF-lacZ* fusion of strain BIV8 in the presence (black bars) or absence (hatched bars) of mitomycin C (100 ng/ml) measured as  $\beta$ -galactosidase activity per OD. The average of two independent experiments is shown.

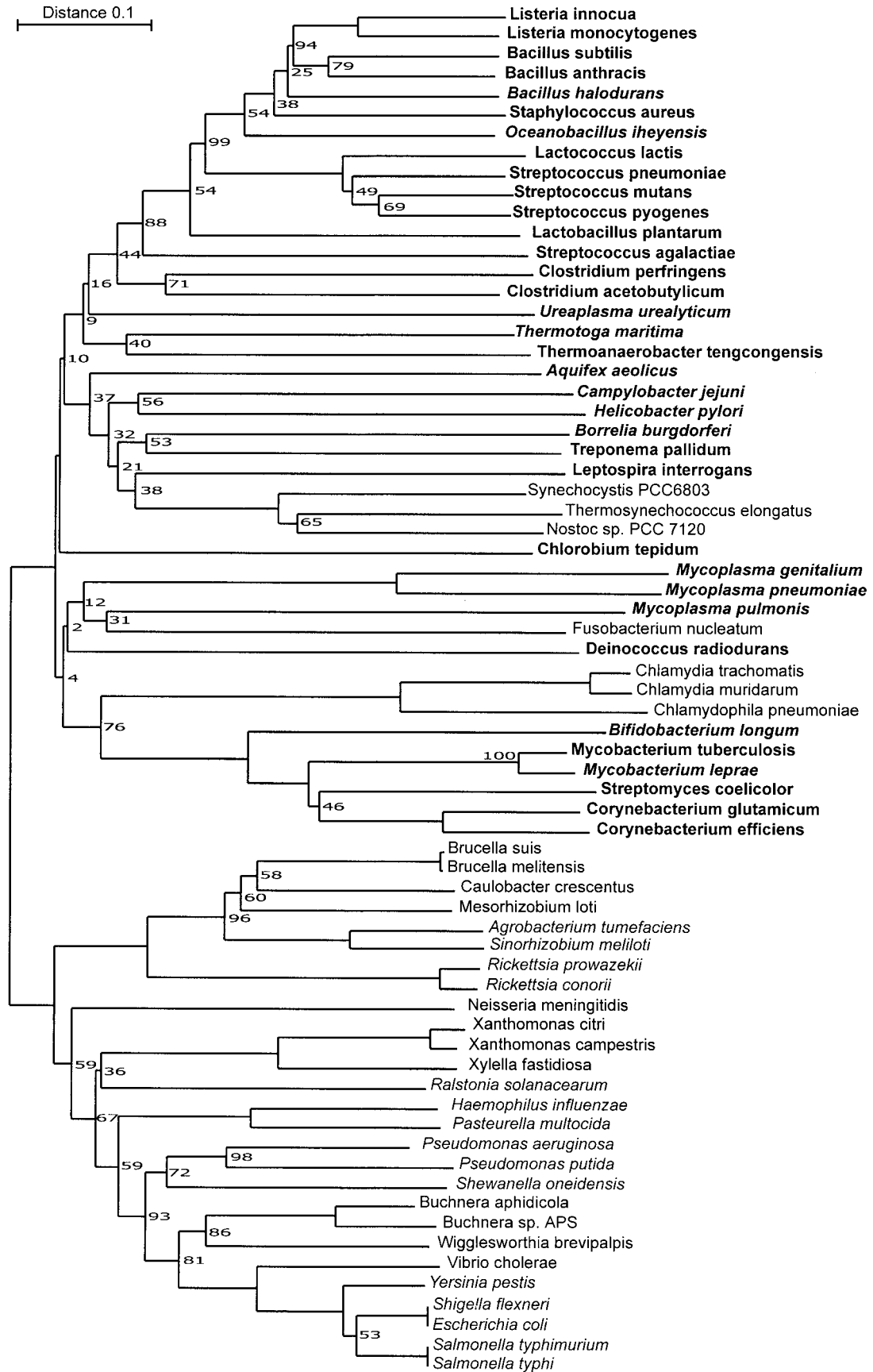
interpreted with care, since no direct protein concentration measurements were carried out.

***ssb* gene is controlled by the SOS response.** It has been reported that the *ssb* gene of *E. coli* is induced by DNA damage (3). However, its DNA damage inducibility is still a matter of discussion (17). *E. coli* SSB is supposed to be involved in the induction of the SOS response by promoting RecA dependent cleavage of LexA (17). Likely SSB serves a similar role in *B. subtilis*. Since *B. subtilis* has two paralogues of SSB that are expressed under different growth conditions, we wondered whether one or both of these SSBs could be induced by DNA-damaging agents too.

When DNA damage was induced by the addition of mitomycin C to the growth medium, an increase of expression of the *ssb* gene from the *rpsF* promoter was observed, starting about 1.5 h after the addition of mitomycin C. A maximum of about threefold elevated expression was reached at 2.5 to 3 h after addition of mitomycin C (Fig. 5). The observed increase of expression is similar to the induction level observed in *E. coli*, which is very slow compared to that of other genes in the *recA-lexA* SOS regulon. Even in the presence of mitomycin C there was no promoter activity detectable from the fragment directly upstream of *ssb* (strain BIV7), confirming the absence of an additional *ssb* promoter (data not shown). These results suggest the involvement of the ribosomal proteins S6 and perhaps also S18 in the SOS response of *B. subtilis*, because their expression is subject to the same control, which mediates the SOS response. Several genes under the control of DinR, the *B. subtilis* LexA homologue, have been identified, and a consensus sequence for its binding to DNA was proposed (30). How-

TABLE 3. Classification based on *ssb* gene organisation

Group	<i>rpsF-ssb-rpsR</i>	<i>uvrA-ssb</i>	Multiple <i>ssb</i> genes	Gram stain	No. of species in group
I	+	-	+	+/-	22
II	+	-	-	+/-	13
III	-	+	-	-	15
IV	-	-	+/-	-	19



ever, no such target sequence could be found in the regulatory region of the *rpsF* gene. Therefore, its SOS-dependent induction might be indirect. From the promoter of *ywpH* no significant mitomycin C-dependent induction could be detected (data not shown).

***ywpH* gene is regulated by ComK and required for optimal competence.** In the wild-type strain induction of *ywpH* expression in MM was observed when exponential growth ceased (Fig. 3). *ywpH* expression reached its maximum 2 h after the onset of stationary phase. This pattern for the regulation of *ywpH* is similar to that of the late competence genes in *B. subtilis*. The development of natural genetic competence is a typical postexponential feature of *B. subtilis* and occurs in response to certain growth conditions such as amino acid limitation in the presence of high glucose concentrations and at high cell density (for reviews see references 8 and 10). The expression of late competence genes is activated by ComK (28). An important step in transformation is recombination, and stable single-stranded DNA is required in this process.

To test the possibility that *ywpH* is regulated by ComK, we introduced a *comK* disruption in *B. subtilis* BIV12, carrying the Y1-*lacZ* fusion. In this *comK* null mutant expression of *ywpH* was totally abolished (Fig. 3), which indicates that *ywpH* belongs to the late competence genes controlled by ComK. In the course of this study we and others also showed by means of transcription analysis of several *B. subtilis* *comK* deletion strains that the transcription of *ywpH* is indeed ComK dependent (2, 12, 19).

We tested the involvement of YwpH in competence. When *ywpH* was disrupted the transformation efficiency dropped approximately 50-fold, although not to zero. In the course of this study Ogura et al. and Berka et al. observed the effect of a *ywpH* disruption on competence within a similar order of magnitude. Competence is not completely annulled in a *ywpH* mutant. This might be due to the presence of the *ssb* gene product which might be able to substitute for YwpH, although only partially.

This result demonstrates an important role of the *ywpH* gene product in competence. In vitro experiments with mutant proteins of the *E. coli* SSB revealed that the C terminus is not required for DNA binding (29). Moreover, the truncated SSB is functional in promoting RecA protein-dependent homologous pairing and strand exchange in vitro (9). Although YwpH is lacking the C terminus present in SSB, it is likely to be able to bind single-stranded DNA and to allow DNA recombination. However, YwpH is probably not able to replace SSB in DNA replication and/or DNA repair, since the C terminus is required for in vivo function (6). In agreement with that, the *ssb* gene was found to be essential for viability in *B. subtilis* (13), whereas the *ywpH* gene could be knocked out without

consequences for bacterial growth under the employed conditions (data not shown). Likely YwpH is involved in homologous DNA recombination processes, which are necessary for acquiring foreign DNA in competent cells of *B. subtilis*. Presumably SSB also can participate in competence-related recombination, since although transformability is greatly reduced, an *ywpH* knockout strain can still be transformed.

**Organization of *ssb* genes in other bacteria.** The *ssb* gene organization in *B. subtilis* differs from the organization observed in *E. coli*. We therefore addressed the question of how common this gene organization is in other bacterial species. For that purpose, genomes of bacteria were screened for SSB homologues and their gene organization with the NCBI database (<http://www.ncbi.nlm.nih.gov>). At this moment 87 complete sequences of bacterial genomes are available, including 69 different species. In all genomes, one or more genes coding for an SSB homologue were found. While only 15 species show the same gene order as in *E. coli* with an *ssb* gene divergently situated to the *uvrA* gene, 35 species show the *ssb* gene flanked by the *rpsF* and *rpsR* genes as it is observed for *B. subtilis*. These 35 species include all gram-positive bacteria sequenced until now, as well as representatives of the *Thermotogales* and *Thermus/Deinococcus* group, and gram-negative species from the phyla *Spirochaetales*, *Aquificales*, *Thermotogales*, and *Chlorobi* and the epsilon subdivision of the proteobacteria. All other proteobacteria and representatives of the *Chlamydiales*, *Cyanobacteria*, and *Fusobacteria* do not possess this gene organization. On the basis of the *ssb* gene and number of *ssb* paralogues per species organization, the sequenced bacteria can be classified in four different groups. An overview is given in Table 3.

Group I contains bacteria with the same *ssb* gene organization as *B. subtilis*, *rpsF-ssb-rpsR*. Most bacteria within this group are Gram positive. All bacteria in this group contain multiple SSB paralogues. Group II contains bacteria with the same organization as *B. subtilis*, *rpsF-ssb-rpsR*, but they do not possess multiple *ssb* paralogues. In addition to some gram-positive bacteria, this group also contains bacteria from the epsilon subdivision of the proteobacteria (*Helicobacter pylori* and *Campylobacter jejuni*), *Ureaplasma urealyticum*, and *Borrelia burgdorferi*. Group III contains bacteria with the same gene organizations as *E. coli*; *ssb* is divergently located to *uvrA*. *Ralstonia solanacearum*, *Salmonella enterica* serovar Typhimurium, *Shewanella oneidensis*, *Pseudomonas aeruginosa*, and *Pseudomonas putida* are also classified within this group. They have one gene located between *uvrA* and *ssb*. All bacteria in this group contain only one *ssb* gene in their chromosome. Furthermore, all these bacteria belong to the alpha or gamma subdivision of the proteobacteria (gram negative). Group IV contains bacteria with *ssb* neither placed between *rpsF* and

FIG. 6. Unrooted phylogenetic tree of bacterial SSBs. Bacterial SSBs from 69 bacteria were used. When bacteria contained multiple *ssb* genes, the *ssb* situated between *rpsF* and *rpsR* was used in this tree. When bacteria have multiple *ssb* genes but do not possess an *ssb* in an operon structure with *rpsF* and *rpsR*, the *ssb* most homologous to the *B. subtilis* *ssb* was used. Alignments were made with ClustalW 1.74 (25) with a gap opening penalty of 30 and a gap extension penalty of 0.5. Dendrogram construction was done with TreeCon 1.3b (27) with the neighbor-joining method and no correction for distance estimation. Bootstrap values (percent) are indicated at the branching points. If no percentage is indicated, the value is 100. The bar indicates 10% difference at the amino acid level. The groups indicated in Table 3 are also indicated in this figure. Bacteria from group I are printed in bold, bacteria from group II are printed in bold italic, bacteria from group III are printed in italic, and bacteria from group IV are printed in normal type.

*rpsR* nor divergently located to *uvrA*. Most of these bacteria contain one *ssb* gene, but *Nostoc* sp. strain PCC 7120 and *Xylella fastidiosa* contain multiple *ssb* genes.

Since we propose a functional division of the gene products from both SSB paralogues, one being the main SSB and the other being competence related, we checked whether the occurrence of multiple SSB homologues in a bacterial genome could be related to natural competence. Most bacteria known to be naturally transformable (14) are classified in group I, which contains species with multiple *ssb* homologues. In group IV only *Nostoc* sp. strain PCC 7120 is known to be naturally transformable, and this species also contains multiple SSBs. The only bacteria that are known to be naturally competent but do not contain multiple *ssb* genes are *H. pylori* and *C. jejuni* (14). These bacteria are both members of the epsilon subdivision of the proteobacteria.

To further investigate if the identified groups can also be divided on the basis of their evolutionary descent, we used all 69 SSB protein sequences to calculate a phylogenetic tree (Fig. 6). The tree shows a clear grouping of groups I and II compared to group III. All the proteobacteria in group IV cluster with the other proteobacteria (group III), but the cyanobacteria, *Chlamydiales*, and *Fusobacterium nucleatum* cluster with the gram-positive bacteria from groups I and II. *Xanthomonas citri* (proteobacteria, gamma subdivision) also clusters outside of the proteobacteria group. Likely this is caused by horizontal gene transfer. In this tree the species from groups I and II are not separated based on their SSB homologies. These results were expected because it is unlikely that the development of the extra SSBs is directly correlated to the sequence of a bacterium's main SSB.

Next to the well-studied *ssb* gene organization observed in *E. coli* and some other gram-negative bacteria, the gene organization *rpsF-ssb-rpsR* observed in *B. subtilis* is a suitable model for the study of *ssb*, since it is found in all gram-positive species sequenced until now and several gram-negative bacteria. Moreover, additional *ssb* genes are found associated with this configuration, frequently in correlation with natural competence.

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