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The *yvyD* Gene of *Bacillus subtilis* Is under Dual Control of σ^B and σ^H

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During a search by computer-aided inspection of two-dimensional (2D) protein gels for σ^B-dependent general stress proteins exhibiting atypical induction profiles, a protein initially called Hst23 was identified as a product of the *yvyD* gene of *Bacillus subtilis*. In addition to the typical σ^B-dependent, stress- and starvation-inducible pattern, *yvyD* is also induced in response to amino acid depletion. By primer extension of RNA isolated from the wild-type strain and appropriate mutants carrying mutations in the *sigB* and/or *spo0H* gene, two promoters were mapped upstream of the *yvyD* gene. The σ^B-dependent promoter drives expression of *yvyD* under stress conditions and after glucose starvation, whereas a σ^H-dependent promoter is responsible for *yvyD* transcription following amino acid limitation. Analysis of Northern blots revealed that *yvyD* is transcribed monocistronically and confirmed the conclusions drawn from the primer extension experiments. The analysis of the protein synthesis pattern in amino acid-starved wild-type and *relA* mutant cells showed that the YvyD protein is not synthesized in the *relA* mutant background. It was concluded that the stringent response plays a role in the activation of σ^H. The *yvyD* gene product is homologous to a protein which might modify the activity of σ^{54} in gram-negative bacteria. The expression of a σ^L-dependent (σ^L is the equivalent of σ^{54} in *B. subtilis*) *levD-lacZ* fusion is upregulated twofold in a *yvyD* mutant. This indicates that the *yvyD* gene product, being a member of both the σ^B and σ^H regulons, might negatively regulate the activity of the σ^L regulon. We conclude that (i) systematic, computer-aided analysis of 2D protein gels is appropriate for the identification of genes regulated by multiple transcription factors and that (ii) YvyD might form a junction between the σ^B and σ^H regulons on one side and the σ^L regulon on the other.

The highly sensitive two-dimensional (2D) protein gel electrophoresis technique combined with the computer-aided evaluation of 2D gels is a very powerful tool for the analysis of the global control of gene expression (1, 51, 59). The transcription of the majority of bacterial genes is organized in regulons that are controlled by global regulators such as repressors, activators, or alternative sigma factors. We used the 2D gel electrophoresis methodology to describe the heat stress stimulon of *Bacillus subtilis*. This heat stress stimulon could be dissected into regulons by searching for proteins that follow the same induction pattern and by analyzing mutants in global regulatory genes (for a review, see reference 20). Increasing attention will be paid to this proteomic approach (59), bearing in mind that a lot of still-unknown regulons were discovered by the sequencing of the *B. subtilis* genome and should be analyzed in the near future (28).

The largest regulon in the heat stress stimulon is the σ^B-dependent general stress regulon of *B. subtilis*, which presumably contains more than 100 genes (4, 5, 20). The function of this regulon was totally unknown until 1994. By the identification of several σ^B-dependent general stress protein genes we and others have obtained evidence that some of these proteins may provide an unspecific, multiple, and prospective general stress resistance to a nongrowing, starving, or stressed *B. subtilis* cell which is no longer able to grow and divide (for a review, see reference 21).

σ^B-dependent stress genes are strongly induced by heat, salt, acid, or ethanol stress as well as by energy depletion (17, 20). The proteins and/or genes belonging to the σ^B regulon follow this typical expression pattern, which can be visualized by a computer-aided evaluation of 2D gels (4). However, we also found general stress proteins which were characterized by a slightly modified induction pattern. In addition to the characteristic σ^B induction pattern the protein YvyD (formerly Hst23), identified by N-terminal sequencing (4), showed a strong induction by amino acid starvation (4, 57). In this paper, we describe this atypical induction profile of *yvyD*. We identified—besides the σ^B-dependent promoter—a second promoter which is recognized by $E\sigma^H$ and which is responsible for the induction of *yvyD* by amino acid starvation. σ^H is used for the transcription of many genes expressed during the transition from exponential growth to the stationary phase (6, 24, 38, 39, 42, 50, 60–63). Such a dual control of a general stress gene by σ^B and σ^H was already described by Varón et al. (52) for the *csb40* operon.

These results show that the 2D protein gel electrophoresis technique is also a useful approach for defining a network of interacting regulons or modulons. We suggest that *yvyD* (and presumably other genes or operons such as *csb40*) may form a junction in a global regulatory network between the σ^B regulon, the σ^H regulon, and most likely the stringent response also.

MATERIALS AND METHODS

Bacterial strains, plasmids, and culture conditions. The bacterial strains and plasmids used in this study are listed in Table 1. *Escherichia coli* DH5α was routinely grown in Luria-Bertani medium and used as the host for cloning experiments (44). *B. subtilis* strains were cultivated with vigorous agitation at 37°C in a synthetic medium described previously (48). For heat shock and osmotic or ethanol stress experiments, exponentially growing cells of *B. subtilis* were shifted from 37 to 48°C or were exposed to either 4% (wt/vol) NaCl or 4% (vol/vol) ethanol. Deprivation of glucose, amino acids, or nitrogen was achieved by cultivating bacteria in the synthetic medium with growth-limiting amounts of glucose (0.05%, wt/vol), amino acids (62.4 μM lysine, 62.4 μM tryptophan), or $(NH_4)_2SO_4$ (1 mM). To generate an artificial amino acid starvation (2, 19), DL-

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TABLE 1. Bacterial strains and plasmids used in this study

Strain or plasmid	Genotype or description	Reference or source
E. coli DH5α	F⁻ F80d*lacZ*ΔM15 Δ(*lacZYA-argF*)*U169 deoR recA1 endA1 hsdR17*(r_K^- m_K^+) *supE44 thi-1 gyrA69*	44
B. subtilis		
IS58 (BR16)	*trpC2 lys-3*	45, 49
IS56 (BR17)	*trpC2 lys-3 relA*	45, 49
BEK38	*trpC2 lys-3 sigB*::ΔHindIII-*Eco*RV::*km*	26a
BKD1	*trpC2 lys-3 yvyD*::*km*	pKD11→IS58[a]
BKD2	*trpC2 lys-3* Δ*spo0H*::*cat*	BH41→IS58
BKD3	*trpC2 lys-3 sigB*::ΔHindIII-*Eco*RV::*km* Δ*spo0H*::*cat*	BH41→BEK38
BKD11	*trpC2 lys-3 amyE*::PΔB(*levD-lacZ*) *cat*	QB5081→IS58
BKD12	*trpC2 lys-3 yvyD*::*km amyE*::PΔB(*levD-lacZ*) *cat*	pKD11→BKD11
QB5081	*trpC2 amyE*::PΔB(*levD-lacZ*) *cat*	30
BH41	*trpC2 pheA1* Δ*spo0H*::*cat*	18
Plasmids		
pBluescript II KS(+)	Cloning vector	Stratagene
pKD1	1.1-kbp fragment of *B. subtilis* DNA containing *yvyD* cloned into pBluescript II KS(+)	This work
pKD2	Internal 0.4-kbp fragment of *yvyD* cloned into pBluescript II KS(+)	This work
pKD11	pKD1 containing a 1,490-bp Km^r cassette from plasmid pGD780 within the *Bgl*II site of *yvyD*	This work
pGD780	Km^r cassette delivery plasmid	16

[a] An arrow indicates the construction of the strain by transformation.

norvaline was added at an optical density at 500 nm (OD_{500}) of 0.5 to a final concentration of 0.05% (wt/vol). *B. subtilis* BKD11 and BKD12 were cultivated in the synthetic medium with 0.2% (wt/vol) glucose (repressing conditions) or with 0.2% (wt/vol) fructose (inducing conditions) (30).

Construction of *B. subtilis* mutant strains. *B. subtilis* BKD2, BKD3, and BKD11 were constructed by transformation of chromosomal DNA from various *B. subtilis* strains into the wild-type strain IS58 or the isogenic *sigB* mutant BEK38. *B. subtilis* BKD1 and BKD12 were constructed by transformation of the wild-type IS58 or BKD11 with the nonreplicative plasmid pKD11. Correct integration was proved by Southern blotting.

Primer extension and Northern (RNA) blot analysis. Total RNA of *B. subtilis* BGH1, BKD2, BKD3, IS58 (BR16), and IS56 (BR17) was isolated from exponentially growing or stressed cells by the acid phenol method described by Majumdar et al. (29) with some modifications (54). The 5′ end of the *yvyD* mRNA was identified by primer extension as described previously (58). The oligonucleotide 5′-CTTCACATCAGCATCCACGC-3′ labelled with [γ-^{32}P] ATP at the 5′ end was used as the primer. Northern blot analysis was performed as described previously (58) with a digoxigenin-labelled RNA probe which was synthesized in vitro with T7 RNA polymerase and the linearized plasmid pKD2 as a template.

Plasmid constructions. The primers P1 (5′-TTGACCAAATTTTTGCGGAG-3′) and P2 (5′-TCATCACACGCCTATTTTAG-3′) were used for the construction of the plasmid pKD1 (see Fig. 1). The resulting PCR product, after amplification of chromosomal DNA of strain IS58, was cloned into pBluescript II KS(+) linearized with *Eco*RV. pKD1 contains the entire *yvyD* gene. The plasmid pKD2 harboring an internal fragment of *yvyD* was constructed in a similar way. The primers P3 (5′-AGTCTAAGGTTGAGGTTACG-3′) and P4 (5′-GGTAC ACGACATTTGTAAGG-3′) were used for the amplification of chromosomal DNA of strain IS58, and the resulting fragment was cloned into pBluescript II KS(+) linearized with *Eco*RV. The plasmid pKD11 was constructed by cloning a 1,490-bp Km^r cassette from plasmid pGD780 (16) within the *Bgl*II site of the *yvyD* gene in the plasmid pKD1.

β-Galactosidase assays. The assay for β-galactosidase activity was performed as described previously (53).

2D polyacrylamide gel electrophoresis. Labelling of cells, 2D polyacrylamide gel electrophoresis, and protein identification on 2D gels were performed as described previously (4, 14).

Computer methods. Alignments were performed with the Genetics Computer Group package at default settings. Databases used were those of the EMBL and GenBank. Figure 2 was generated by the program BOXSHADE.

General methods. Plasmid isolation, restriction enzyme analysis, transformation of *E. coli*, ligation of DNA fragments, and filling in of 3′ termini with Klenow fragments of DNA polymerase I were performed according to standard protocols (44). Chromosomal DNA from *B. subtilis* was isolated with a genomic DNA purification kit (Promega). Transformation of *B. subtilis* was carried out according to the method described by Hoch (22).

RESULTS

Identification of Hst23 as the product of the *yvyD* gene. YvyD (Hst23) has been described as a general stress protein of *B. subtilis* (4). In 2D protein gels, YvyD is characterized by the typical σ^B-dependent stress and starvation induction pattern; however, in contrast to most products of the σ^B-dependent general stress genes YvyD is also induced by amino acid starvation (57).

The sequence encoding the N terminus of YvyD shows 100% identity to *orf189*, which is located between the *fli* operon and the *secA* gene in *B. subtilis* (7) (Fig. 1). A computer-aided search for similarities with other proteins showed an identity of 30 to 40% between this open reading frame encoding a 189-amino-acid polypeptide and σ^{54} modulating factors of various gram-negative bacteria (33) (Fig. 2).

During the *B. subtilis* genome sequencing project, *orf189* was renamed *yvyD* (28). In this paper, we refer to the gene encoding Hst23 (Orf189) as *yvyD*.

Mapping of the *yvyD* promoters and Northern blots. The transcriptional regulation of *yvyD* was analyzed by primer ex-

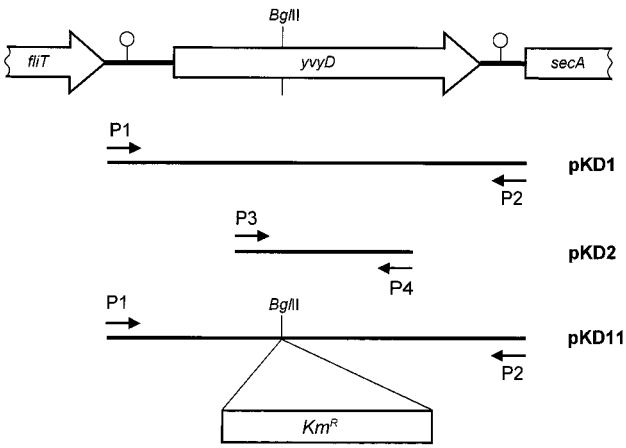


FIG. 1. Schematic representation of the *yvyD* region in the chromosome of *B. subtilis*. The *yvyD* gene is located between the *fli* operon and the *secA* gene. A rho-independent terminator is located upstream and downstream of *yvyD* (7). The locations of the primer pair P1-P2, used for construction of plasmid pKD1, and the primer pair P3-P4, used for the construction of plasmid pKD2, are indicated. The *Bgl*II site within *yvyD* was used for the construction of the *yvyD* mutant by the insertion of a kanamycin resistance cassette in plasmid pKD1, resulting in plasmid pKD11.

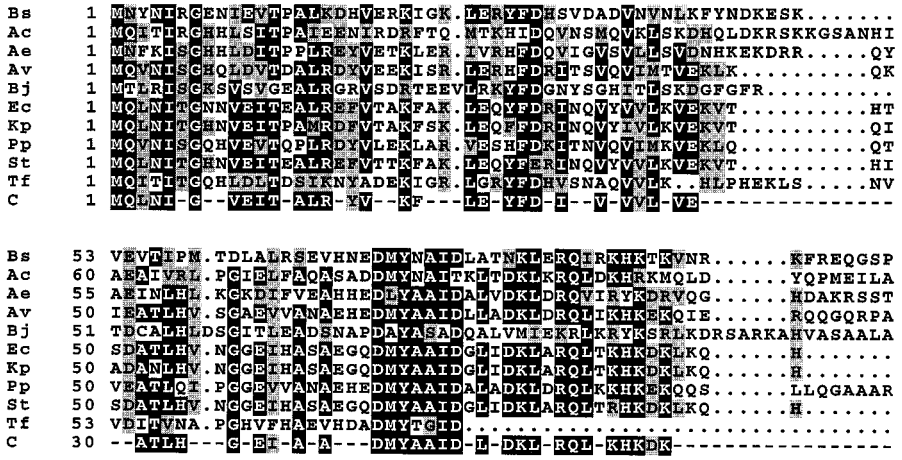
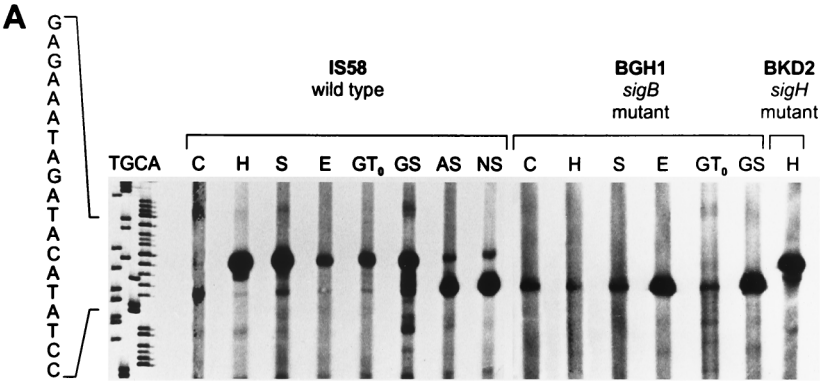


FIG. 2. Multiple, partial alignment (approximately 100 amino acids at the N terminus) of YvyD with proteins functioning as σ^{54} modulating factors from gram-negative bacteria. Bs, *B. subtilis*; Ac, *Acinetobacter calcoaceticus* (*orf2*) (10); Ae, *Alcaligenes eutrophus* (*Ralstonia eutropha*) (*orf130*) (55); Av, *Azotobacter vinelandii* (second open reading frame) (32); Bj, *Bradyrhizobium japonicum* (*orf203*) (27); Ec, *E. coli* (*orfII*) (25); Kp, *K. pneumoniae* (*orf95*) (33); Pp, *Pseudomonas putida* (*orf102*) (26); St, *Salmonella typhimurium* (36); Tf, *Thiobacillus ferrooxidans* (*orf3*) (3); C, consensus sequence.

tension (Fig. 3) and Northern blotting (Fig. 4). By the primer extension technique two transcriptional start sites, separated by 5 nucleotides, were found. The potential −10 and −35 regions of the upstream promoter are similar to those of known σ^B-dependent genes. The downstream promoter revealed high similarities to promoters recognized by RNA polymerase containing σ^H. In exponentially growing cells of the wild-type strain IS58, transcription is mainly initiated at the σ^H-dependent promoter (Fig. 3). In a *sigH sigB* double mutant there is no transcription of *yvyD* at all, supporting the hypothesis that only σ^B and σ^H are involved in transcriptional regulation (Fig. 4). Northern blot analyses showed that *yvyD* is transcribed monocistronically. A 600-bp signal was detected as the main transcript (Fig. 4). Both sigma factors contribute to the expres-



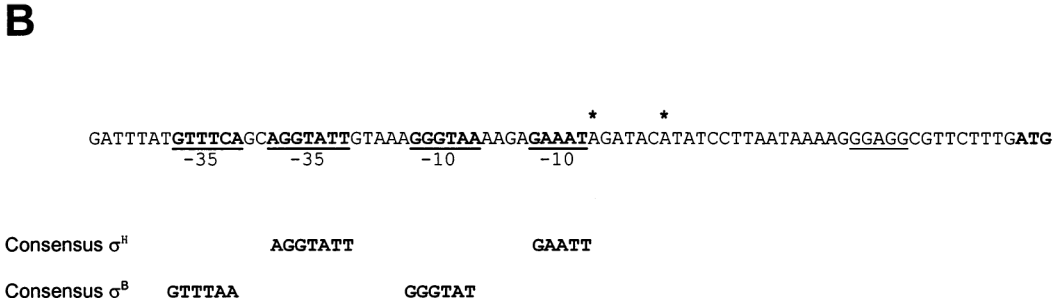


FIG. 3. Determination of the transcriptional start sites of *yvyD*. (A) Primer extension analysis of RNA. The *B. subtilis* wild-type strain IS58, the *sigB* mutant strain BGH1, and the *sigH* mutant strain BKD2 were exposed at an OD_{500} of 0.5 to various stresses as described in Materials and Methods. For RNA isolation, bacteria were harvested before exposure (C, control) and 6 min after exposure to the different stressors (H, heat [48°C]; S, salt [4% NaCl]; E, ethanol [4% ethanol]). In cases of nutrient starvation bacteria were harvested at transient phase (glucose depletion [GT_0]) or 30 min after entry into stationary phase (deprived of glucose [GS], amino acids [AS], or nitrogen [NS]). A total of 5 μg of each RNA preparation was used for each primer extension reaction. Lanes T, G, C, and A show the sequencing ladder obtained with the same primer as that used for primer extension. (B) DNA sequence of the *yvyD* promoter region. Potential start sites are indicated by asterisks. The σ^B- and σ^H-dependent promoters are printed in bold. The consensus sequence for σ^B-dependent promoters is taken from the work of Hecker et al. (20), and the consensus sequence for σ^H-dependent promoters is derived from the work of Haldenwang (17). The ribosomal binding site is underlined, and the translational start codon is shown in bold.

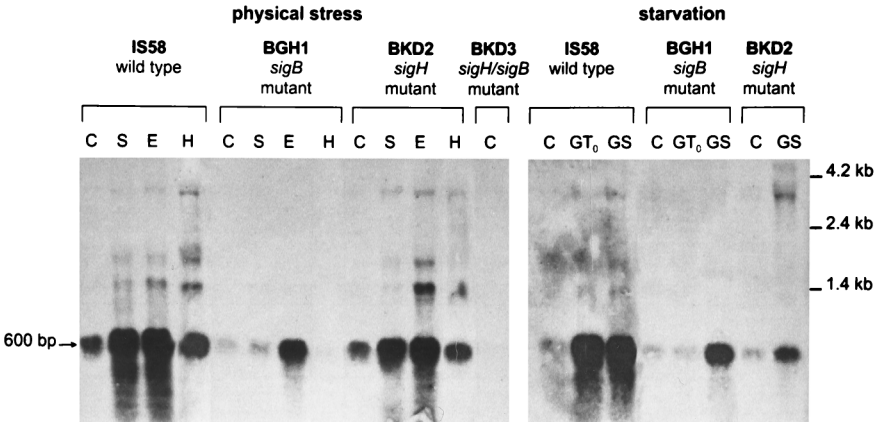


FIG. 4. Northern blot analysis of stress-inducible *yvyD* transcription. Total RNA was isolated from *B. subtilis* IS58, BGH1, BKD2, and BKD3 after exposure to various stress conditions as described in the legend to Fig. 3. A total of 5 μg of RNA was applied in each lane. The locations of RNA molecular size markers and the size of the *yvyD* transcript are marked. C, control; S, salt; E, ethanol; H, heat; GT_0, glucose depletion at transient phase; GS, glucose depletion at stationary phase.

sion pattern. In a σ^H mutant the transcription of *yvyD* depends solely on $E\sigma^B$, showing the typical induction profile for σ^B-dependent genes: *yvyD* is strongly induced by heat, salt, or ethanol stress as well as by glucose starvation (Fig. 3 and 4). In a σ^B mutant, however, no heat stress induction occurred at the σ^H-dependent promoter, but an induction by glucose starvation (Fig. 3 and 4) and another by amino acid starvation (data not shown) did. However, the transcription at the σ^H-dependent promoter appeared to show a delayed induction in response to starvation. Surprisingly, we also found a σ^H-dependent induction by ethanol treatment. Concerning the transcriptional regulation of *yvyD*, we note that the induction by physical stress and glucose starvation depends on σ^B and that the induction by amino acid starvation depends solely on σ^H (Fig. 3). When both sigma factors are activated (by ethanol stress and glucose starvation) $E\sigma^B$ most probably is involved to a larger extent in the transcription of *yvyD* than $E\sigma^H$. However, the possibility that there really is a competition between both sigma factors for the transcription of *yvyD* needs further investigation.

YvyD induction by amino acid starvation in the *relA* mutant. The synthesis of YvyD was strongly stimulated by norvaline treatment, which triggers a stringent response via leucine and isoleucine limitation (2, 19). In accordance with the findings of Wendrich and Marahiel (57), this induction did not occur in a *relA* mutant (Fig. 5). Figure 5 also demonstrates that the ribosomal protein RplJ is subjected to the characteristic stringent response, i.e., the continued synthesis in the *relA* mutant versus downregulated synthesis in the wild type. Transcriptional studies by Northern blotting provided additional evidence that *yvyD* is strongly induced by amino acid depletion at the σ^H-dependent promoter only in the wild type and not in the *relA* mutant (Fig. 6).

Analysis of σ^L-dependent gene expression in a *yvyD* mutant. σ^L in *B. subtilis* is the equivalent of σ^{54} in gram-negative bacteria (9). Because it had been shown that σ^{54}-dependent transcription was elevated by 25% in a strain of *Klebsiella pneumoniae* carrying a mutation in the gene homologous to *yvyD* (*orf95*) (33), we examined the transcription of the σ^L-depen-

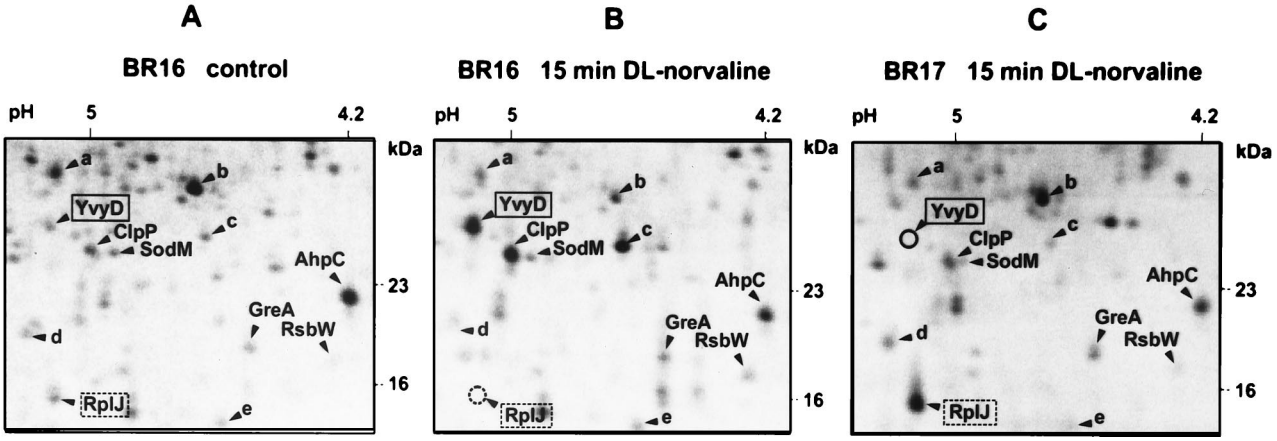


FIG. 5. Sections of 2D protein gels in the YvyD region. Strains BR16 (wild type) and BR17 (*relA*) were grown to an OD_{500} of 0.5. For panels B and C an artificial depletion of amino acids was generated by the addition of DL-norvaline to a final concentration of 0.05%. Labelling of cells with [^{35}S]methionine (5 μCi/ml) was performed after 15 min of incubation with DL-norvaline for 3 min. (A) Control (strain BR16, exponentially growing cells, labelling at an OD_{500} of 0.5, and no DL-norvaline addition); (B) strain BR16 after DL-norvaline treatment; (C) strain BR17 after DL-norvaline treatment. The locations of the proteins ClpP, SodM, GreA, AhpC, and RsbW and of the unidentified proteins a, b, c, d, and e are included as references. Note in panel C that the YvyD protein is no longer induced in the *relA* mutant and that the protein RplJ is not subjected to the stringent response.

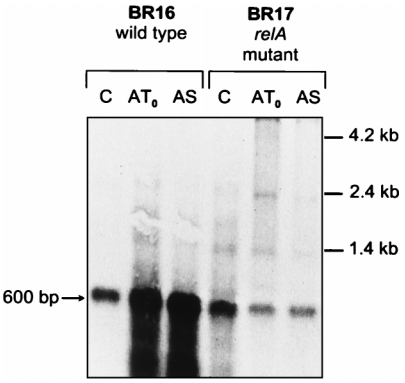


FIG. 6. Northern blot analysis of stress induction of *yvyD* transcription in a *relA* mutant. Total RNA was isolated from *B. subtilis* BR16 and BR17 before (C, control) and after depletion of amino acids (AT_0, transient phase; AS, 30 min after entry into stationary phase). A total of 2.5 μg of RNA was applied in each lane. The locations of RNA molecular size markers and the size of the *yvyD* transcript are marked. Note that induction of *yvyD* transcription does not occur in the *relA* mutant strain.

dent *lev* operon in *B. subtilis*. The *lev* operon is induced by fructose and repressed by glucose in the medium (30).

The β-galactosidase activity originating from a *levD-lacZ* fusion was measured in the wild-type strain BKD11 and in the *yvyD* mutant strain BKD12. In the presence of fructose the expression of *levD-lacZ* was elevated twofold in the *yvyD* mutant relative to that in the wild type (Fig. 7). The relative amount of the *sigL* transcript is not increased in the *yvyD* mutant relative to that in the wild type as revealed by slot blot analysis (data not shown). These results indicate that YvyD might influence the activity of σ^L in *B. subtilis*.

DISCUSSION

By a careful and comprehensive computer-aided inspection and matching of various 2D gels loaded with radioactively labelled proteins from growing, starved, or stressed *B. subtilis* cells, it is possible to proceed from a 2D protein index to a more global analysis and description of the gene expression network (1). The proteomic research approach (59) relying mainly on the highly sensitive 2D protein gel electrophoresis technique is a useful approach not only for the definition of stimulons and regulons (20, 51) but, as shown in this study, also for the discovery and preliminary analysis of genes controlled by more than one regulatory circuit.

σ^B-dependent general stress genes show an expression pattern induced by heat, salt, acid, or ethanol stress on the one hand and by glucose, oxygen, or phosphate starvation on the other. During a search for general stress proteins exhibiting atypical induction profiles we found YvyD, which shows the typical σ^B-dependent induction pattern. However, the strong induction of YvyD caused by amino acid starvation did not fit with the σ^B-dependent induction profile. In this paper it is shown that in addition to the σ^B promoter, *yvyD* contains a second, σ^H-dependent promoter responsible for this atypical induction profile. Our results indicate that a presumably small subset of σ^B-dependent genes are also controlled by σ^H, extending the inducing environmental stimuli to amino acid or nitrogen starvation. The first member of this σ^B-σ^H modulon was described by Varón et al. (52), who found the *csb40* operon to be under this dual control. The second open reading frame of the *csb40* operon (*orf2*) showed a significant resemblance to desiccation proteins occurring in dried leaves of plants. The description of a new member of this stationary-phase or general stress modulon seems to support the conclusion of Varón et al. (52) that σ^H may be more broadly involved in stress response than previously suggested. Very recently, Gaidenko and Price (13) found σ^H to be involved in the stress resistance of *B. subtilis*. However, the number of genes whose expression is controlled by both sigma factors seems to be rather low, because from about 30 to 40 general stress proteins whose expression profiles were analyzed by a computer-aided evaluation, only Hst23 showed this atypical induction pattern (4).

The σ^H regulon and the expression of the *spo0H* gene have been intensively investigated. σ^H has been described as a sigma factor responsible for the transcription of several genes expressed at the transient phase at the start of sporulation and competence development (for reviews, see references 15 and 23). Several genes under σ^H control, which are expressed early in the transition period, also possess a σ^A-dependent promoter; some genes of the σ^H regulon are not directly involved in the sporulation pathway, e.g., *citG*, *rpoD*, and the *ureABC* operon (6, 39, 50, 60). Early-sporulation genes, *spo0A*, *spo0F*, *kinA*, *spoVG*, and *spoVS*, are transcribed by $E\sigma^H$ (11, 38, 42, 43, 63). The *spoIIA* operon, encoding the prespore- and forespore-specific sigma factor σ^F, is transcribed exclusively by $E\sigma^H$ (61, 62). The expression of *spo0H* itself increases as the culture enters the late logarithmic stage of growth (56). An acidification of the internal pH negatively influences *spo0H* expression (8). The decreasing level of the transition state regulator AbrB, whose synthesis is repressed by phosphorylated Spo0A, seems to be responsible for the transient derepression of *spo0H* (11, 35, 46, 47, 56, 63).

We noticed some interesting details about the expression of *yvyD*. Our transcriptional data appeared to show that in response to glucose starvation the σ^B-dependent promoter is activated earlier than the σ^H-dependent one. Furthermore, the σ^H-dependent induction of *yvyD* by ethanol in the *sigB* mutant background is a result which deserves future attention. The most promising result, however, is that in a *relA* mutant *yvyD* is

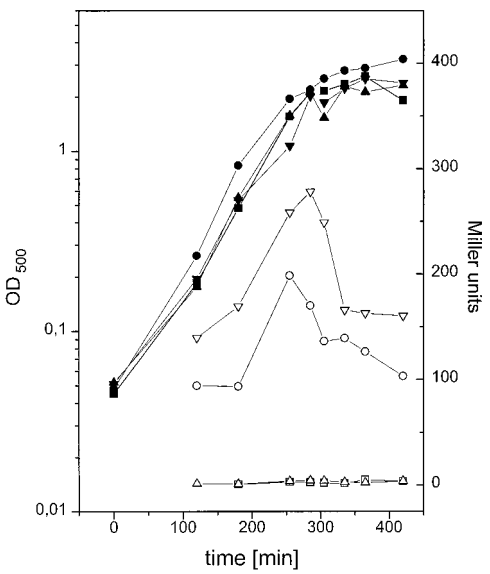


FIG. 7. β-Galactosidase synthesis originating from a *levD-lacZ* fusion in the wild type and the *yvyD* mutant. Strains BKD11 (*levD-lacZ*) and BKD12 (*levD-lacZ yvyD*) were grown in minimal medium containing either 0.2% glucose (repressing conditions) (BKD11 [■] and BKD12 [▲]) or 0.2% fructose (inducing conditions) (BKD11 [●] and BKD12 [▼]) as the carbon source. At the times indicated, samples were removed and assayed for β-galactosidase activity (corresponding open symbols).

no longer induced in response to amino acid starvation. The *relA*-dependent induction of *yvyD* occurs at the σ^H-dependent promoter because σ^B-dependent genes are not induced after ppGpp accumulation (31). An intriguing explanation for this result could be that σ^H requires the stringent response for its activity regardless of the mechanism of this proposed activation. It is tempting to speculate that ppGpp is somehow involved in the derepression of *spo0H*. However, several reports indicate that *spo0H* expression is regulated at different levels of gene expression, including even the posttranslational level (12, 18, 41, 56). Recently, it has been found that YvyD is accumulated in *clpP* and *clpX* mutants (14). This observation can be explained by the recent finding that σ^H is a substrate for Clp proteases (34). Further studies are necessary to elucidate the still-putative relationship between SigH activity and the stringent response.

The function of YvyD is still unknown. The protein shows 30 to 40% identity to σ^{54} modulating factors of gram-negative bacteria; the *yvyD* gene is transcribed monocistronically. In contrast to *B. subtilis*, the genes encoding the σ^{54} modulating factors are organized in operons coding for σ^{54}-like factors (3, 10, 25–27, 32, 33, 36, 55) and for proteins highly similar to components of the phosphotransferase system (37) in gram-negative bacteria. Reizer et al. (40) proposed that the phosphotransferase system catalyzed protein phosphorylation functions in the regulation of σ^{54}-dependent gene expression and supposed a link between carbon and nitrogen metabolic pathways. The σ^{54} modulating proteins do not seem to act as repressors but may inhibit the activity of the sigma factor via a still-unknown mechanism (33). In a *yvyD* mutant of *B. subtilis*, transcription of the σ^L-dependent operon *levD* seems to be elevated, indicating that YvyD may also exert a negative effect on σ^L activity. Despite the fact that we do not yet know the physiological role of YvyD, the outstanding location of *yvyD* in the gene regulation network linking σ^B and σ^H on the one side with σ^L on the other might promise a very important function of this protein in the genetic network of the transition phase which needs further investigation.

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