Identification and transcriptional analysis of new members of the σ^B regulon in *Bacillus subtilis*

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***Bacillus subtilis* responds to various stimuli (heat, ethanol and salt stress, energy starvation) with the induction of general stress proteins (GSPs). Most of them belong to the stress and stationary-phase regulon controlled by the alternative sigma factor σ^B . The majority of σ^B -dependent proteins are thought to provide a precautionary general stress resistance in stressed or starved cells. In this report, the identification and transcriptional analysis of nine new members of the σ^B regulon are described. The biochemical function was not determined for any of the proteins encoded by the nine new σ^B -dependent stress genes, however, similarities to proteins in the databases allowed a distinction between proteins with putative (i-iv) and unknown (v) function. The putative functions of BmrU, YcdF, YdaD, YdaP, YhdN and YocK underline the suggested protective role of σ^B -dependent GSPs and also elucidate new areas where σ^B might play an important role. (i) The finding that the *bmrUR* operon is under σ^B control indicates that the elimination of multidrug compounds might be a new function in multiple stress resistance. (ii) YcdF and YdaD resemble NAD(P)-dependent dehydrogenases. Both proteins could be involved in the generation of NAD(P)H and therefore in the maintenance of the intracellular redox balance under stress. (iii) The *ydaP* gene might belong to the increasing number of σ^B -dependent genes whose orthologues are under the control of σ^S in *Escherichia coli*, indicating that both regulons may fulfil similar functions. (iv) YhdN shows weak similarities to potassium ion channel proteins and YocK shows resemblance to the DnaK suppressor protein DksA. (v) Three new σ^B -dependent genes (*ydaE*, *ydaG* and *yfkM*) encoding proteins with still unknown functions were also described. Further analyses of corresponding mutants might allow a first prediction of their function within the framework of the general stress regulon.**

Keywords: *B. subtilis*, new σ^B -dependent genes

INTRODUCTION

As revealed by two-dimensional (2-D) PAGE analyses, more than 60 general stress proteins (GSPs) are induced after heat shock, ethanol and salt stress or after glucose starvation in *Bacillus subtilis* (Antelmann *et al.*, 1997a; Bernhardt *et al.*, 1997). Many genes encoding GSPs are controlled by the alternative stress and stationary-phase sigma factor σ^B (Haldenwang & Losick, 1980; Boylan *et al.*, 1993a; Haldenwang, 1995; Hecker *et al.*, 1996).

Despite a good understanding of the complex signal transduction pathway that controls the activity of σ^B (Benson & Haldenwang, 1992, 1993a, c; Boylan *et al.*, 1992; Völker *et al.*, 1995a, b, 1996; Wise & Price, 1995; Alper *et al.*, 1996; Yang *et al.*, 1996; Akbar *et al.*, 1997) and a large number of known σ^B -dependent stress genes, the function of σ^B has long been a matter of speculation (Binnie *et al.*, 1986; Duncan *et al.*, 1987). Because of the typical induction profile (Benson & Haldenwang, 1993b; Boylan *et al.*, 1993b; Völker *et al.*, 1994), σ^B -dependent stress proteins were thought to provide a prospective multiple stress resistance to non-growing cells as the Pex (post-exponential starvation) proteins do in *Escherichia coli* (Matin, 1991). This assumption was

Abbreviations: 2-D, two-dimensional; GSP, general stress protein.

Table 1. Plasmids and primers used in this study

Plasmid or primer	Characteristics or sequence (5'–3')	Source
Plasmids		
pBluescript II KS(+)	Cloning vector; Ap ^r	Stratagene
pBluescript II KS(–)	Cloning vector; Ap ^r	Stratagene
pKS(+)-bmrU	pBluescript II KS(+) containing the 1055 bp <i>bmrU</i> PCR fragment	This study
pKS(+)-ydaD	pBluescript II KS(+) containing the 1105 bp <i>ydaD</i> PCR fragment	This study
pKS(+)-ydaE	pBluescript II KS(+) containing the 356 bp <i>ydaE</i> PCR fragment	This study
pKS(+)-ydaP	pBluescript II KS(+) containing the 1838 bp <i>ydaP</i> PCR fragment	This study
pKS(–)-yhdN	pBluescript II KS(–) containing the 1131 bp <i>yhdN</i> PCR fragment	This study
pKS(–)-yocK	pBluescript II KS(–) containing the 532 bp <i>yocK</i> PCR fragment	This study
Primers*		
bmrU-forward	CGGGATCCCGCTTTGCTTCGCCGTGCAC	This study
bmrU-reverse	CCCAAGCTTGGGCTAGGGGAGAGTCAACGG	This study
bmrU-PE	CTTTGTCTGTTTAATAATCACCTCATC	This study
ydaD-forward	CGGGATCCCGAATCATGCCGATGACGCC	This study
ydaD-reverse	CCCAAGCTTGGGGCCCATATGTCTCACTCC	This study
ydaD-PE	CTGCTCCTTTATAATCCTCGT	This study
ydaE-forward	CGGGATCCCGCAGCTCTTCGTCTACGTC	This study
ydaE-reverse	CCCAAGCTTGGGTATCCGTCGTCGTCGATG	This study
ydaG-forward	CGGGATCCCGTCCCCGGATCTGGAAAAG	This study
ydaG-reverse	GAAGCGTAAGCTTCTCTCTC	This study
ydaG-PE	GTAATCGTCAGCCCGTCATGG	This study
ydaP-forward	CGGGATCCCGGTGATTACAGCTCTCCGGG	This study
ydaP-reverse	CCCAAGCTTGGGAACAAGGCCTCTTAGGGC	This study
ydaP-PE	CCGCTACCTCTTCGTGGCGG	This study
yhdN-forward	CCCAAGCTTGGGTTTCATCGATCGTCACTGCC	This study
yhdN-reverse	CGGGATCCCGCTTCACTGTTAGCGTCC	This study
yocK-forward	CCCAAGCTTGGGTGCCAACGAAAACCCGAAGC	This study
yocK-reverse	CGGGATCCCGGAAGGAGCATCTGTCTCCAG	This study

* The restriction sites of the restriction enzymes *Bam*HI (5' GGATCC 3') and *Hind*III (5' AAGCTT 3') are underlined.

supported by the finding that the orthologues of the σ^B -dependent genes *katE*, *dps*, *opuE* and *osmC* (Engelmann *et al.*, 1995; Antelmann *et al.*, 1997b; von Blohn *et al.*, 1997; Völker *et al.*, 1998) were under the control of the stress and starvation sigma factor σ^S in *E. coli* (Almiron *et al.*, 1992; Altuvia *et al.*, 1994; Loewen & Hengge-Aronis, 1994; Mellies *et al.*, 1995; Schellhorn, 1995; Gordia & Gutierrez, 1996).

One essential function of the σ^B -dependent general stress response is to provide resistance against oxidative stress in stationary-phase cells of *B. subtilis*. This requires the presence of the non-specific DNA-binding and -protecting protein Dps in *B. subtilis* (Antelmann *et al.*, 1997b). A *dps* deletion mutant fails to develop starvation-induced non-specific resistance to lethal concentrations of hydrogen peroxide (Antelmann *et al.*, 1997b). Other products of σ^B -dependent genes such as KatE and TrxA, as well as Sms and YacK encoded by the fifth and sixth genes of the *clpC* operon, may also be involved in the protection from oxidative damage of membranes, proteins or DNA (Engelmann *et al.*, 1995; Krüger *et al.*, 1997; Scharf *et al.*, 1998). σ^B -dependent proteins also seem to participate in the adaptation to acid, alkaline, osmotic and heat stress, including the

renaturation or degradation of aberrant proteins (Krüger *et al.*, 1994; Gaidenko & Price, 1998; Gerth *et al.*, 1998; Spiegelhalter & Bremer, 1998).

In summary, the products of σ^B -dependent general stress proteins are expected to provide a wide ranging and prospective stress resistance to non-growing *B. subtilis* cells in anticipation of future stress (for a review see Hecker & Völker, 1998). To give additional evidence for these proposed functions and to understand the physiological role of the entire σ^B regulon, new σ^B -dependent genes were identified and their expression was studied.

METHODS

Bacterial strains and culture conditions. The bacterial strains used in this study were *B. subtilis* 168 (*trpC2*; Anagnostopoulos & Spizizen, 1961), ML6 (*trpC2 sigB::ΔHindIII-EcoRV::cat*; Igo *et al.*, 1987) and *E. coli* DH5 α (Hanahan, 1983). DH5 α was routinely grown in a complex medium and used as the host for transformation and propagation of plasmids. *B. subtilis* strains were cultivated under vigorous agitation at 37 °C in a synthetic medium containing 15 mM (NH₄)₂SO₄, 8 mM MgSO₄·7H₂O, 27 mM KCl, 7 mM sodium citrate·2H₂O, 50 mM Tris/HCl pH 7.5, supplemented with 0.6 mM KH₂PO₄, 2 mM CaCl₂·2 H₂O,

1 μ M $\text{FeSO}_4 \cdot 7\text{H}_2\text{O}$, 10 μ M $\text{MnSO}_4 \cdot 4\text{H}_2\text{O}$, 4.5 mM glutamic acid, 780 mM tryptophan and 0.2% glucose (w/v) (Stülke *et al.*, 1993). For heat stress, the cells were shifted from 37 °C to 48 °C. The other stress conditions were achieved by exposing cells to either 4% (w/v) NaCl or 4% (v/v) ethanol. Starvation for glucose was provoked by cultivating the bacteria in minimal medium containing limiting amounts of glucose [0.05% (w/v)].

Analytical and preparative 2-D PAGE and N-terminal sequencing. Analytical 2-D PAGE was performed with bacterial cell extracts, which were labelled with 5 μ Ci L-[^{35}S]methionine (ml culture) $^{-1}$ (NEN DuPont; specific activity > 1000 Ci mmol $^{-1}$; 1 μ Ci = 37 kBq) for 3 min before (control) and 10 minutes after exposure to ethanol stress, using the immobilized pH gradient (IPG) technique and the protocols provided by Pharmacia. For N-terminal sequencing, protein samples were separated by preparative 2-D PAGE and the protein spots collected from several 2-D gels were concentrated, blotted and sequenced as described by Schmid *et al.* (1997).

Analysis of transcription. Total RNA of the *B. subtilis* strains was isolated from exponentially growing cells before (control) and after the exposure to stress or after glucose starvation using the acid phenol method (Majumdar *et al.*, 1991) with modifications as previously described (Völker *et al.*, 1994).

For Northern-blot analyses, equal amounts of total RNA (1–10 μ g) were separated through a 1.5% formaldehyde-MOPS gel and blotted onto a positively charged nylon membrane (Boehringer Mannheim). Hybridizations specific for the genes *bmrU*, *ydaD*, *ydaE*, *ydaG*, *ydaP*, *yhdN* and *yocK* were conducted with digoxigenin-labelled antisense RNA probes synthesized *in vitro* using either T3 or T7 RNA polymerase from *Bam*HI- or *Hind*III-linearized recombinant plasmids, respectively. Non-radioactive hybridization and detection were performed according to the manufacturer's instructions (Boehringer Mannheim). The genes were amplified from chromosomal DNA of *B. subtilis* 168 using the restriction-site-containing PCR primers shown in Table 1.

For primer-extension experiments, the 5' end of each synthetic oligonucleotide primer (100 ng μ l $^{-1}$), which is complementary to the N-terminal coding region of the respective gene, was labelled with 50 μ Ci [γ - ^{32}P]ATP (NEN DuPont; specific activity > 6000 Ci mmol $^{-1}$) and used as a primer for cDNA synthesis as described by Wetzstein *et al.* (1992). DNA sequencing of the corresponding promoter regions was performed according to Sanger *et al.* (1977) using the recombinant plasmids listed in Table 1.

General methods. Cloning of PCR products, plasmid isolation, restriction enzyme analyses, ligation of DNA fragments and transformation of *E. coli* were conducted according to standard protocols (Birnboim & Doly, 1979; Sambrook *et al.*, 1989; Harwood & Cutting, 1990; Ausubel *et al.*, 1995). Database searches were performed using the BLAST program (version 2.0; Altschul *et al.*, 1997).

RESULTS

Expression of six new genes described here is shown to be dependent on σ^B from 2-D gel analysis and protein microsequencing of their products (YcdF = Gsp74, YdaD = Gsp39, YdaG = Gsp26, YfkM = Gsp18, YhdN = Gsp69, YocK = Gsp16u; see Fig. 1); these GSPs were no longer induced in a *sigB* mutant by stress or

starvation for glucose (Völker *et al.*, 1994; Antelmann *et al.*, 1997a; Bernhardt *et al.*, 1997). For most of these new genes we first confirmed their σ^B dependence by Northern analyses, then found consensus σ^B -recognition sequences preceding them. These results strongly suggest that the new genes are directly transcribed by σ^B -containing RNA polymerase *in vivo*. For a subset of these genes (*ydaDE* and *ydaG*), we further demonstrated by primer extension experiments that the 5' end of their messages lay 9–10 nt downstream of the proposed σ^B -recognition sequences. The σ^B dependency of *bmrU* and *ydaP*, which show typical σ^B promoters, was also confirmed by Northern analyses as well as primer-extension experiments. Lastly, for two of the new genes (*ycdF* and *yfkM*), we conducted no additional experimental work beyond the 2-D gel analysis, but we did note typical σ^B -recognition sequences preceding their coding regions. Genetic and biochemical analyses of the function of these newly described GSPs were beyond the scope of this study. Therefore, physiological functions were not defined for any of the nine new proteins. However, similarities to proteins in the databases may indicate putative functions for six of them. The proteins were listed in two main groups with putative (i–iv) and unknown function (v) in alphabetical order by the current gene designation.

(i) The *bmr* locus had already been sequenced and characterized by Ahmed *et al.* (1994) and is located at 212.9° on the *B. subtilis* chromosome. Proteins encoded by the *bmrUR* operon such as Bmr (transporter) and BmrR (regulator) are involved in multidrug resistance (Ahmed *et al.*, 1994). However, BmrU, which is also part of the operon, showed no significant similarity to any known protein in the databases.

Transcription of *bmrU* was very low during exponential growth but increased severalfold after heat stress, exposure to ethanol and salt as well as after glucose starvation. Several transcripts were detected after stress in wild-type cells but not in the *sigB* mutant (Fig. 2a, b). The *bmrU* gene was transcribed as a monocistronic mRNA (about 1.0 kb) as well as cotranscribed with either *bmr* (about 2.5 kb) or with *bmr* and *bmrR* (approx. 3.8 kb) as earlier suggested by Neyfakh (see accession no. L25604) (Fig. 2b). Since the *yqiW* gene, which precedes the *bmrUR* operon, is transcribed in the opposite direction it should be expected that *bmrU* is not cotranscribed with *yqiW*. A σ^B -dependent promoter consensus sequence (GTTTAC-N₁₄-GGGGAT) was found in front of *bmrU* and the corresponding transcriptional start site of the RNA polymerase was detected by primer extension experiments in wild-type but not in *sigB* mutant cells (Fig. 2c).

(ii) The YcdF protein (Gsp74; Fig. 1a, b), which is similar to *Bacillus megaterium* glucose 1-dehydrogenases (Table 2), showed the typical σ^B -dependent induction profile and a σ^B -consensus promoter sequence in front of the *ycdF* gene (GTTTTC-N₁₄-GGGTAT).

The translated amino acid sequence of *ydaD* showed a similarity to proteins encoded by the paralogous genes

Fig. 1. For legend see facing page.

Fig. 2. Schematic representation of the chromosomal region (a), the results of Northern blot analyses (b) and the mapping of the transcriptional start sites by primer extension experiments (c) for the *bmrU* gene. The RNA probe used for the Northern blot analysis is indicated as a grey bar. The detected σ^B -dependent transcripts are shown as black arrows below the probe. The transcriptional-start site is marked with an asterisk in (c). The σ^B -promoter sequence is underlined, and the transcriptional- and the translational-start sites are in bold. Northern-blot analyses and primer extension experiments were conducted as described in Methods using RNA isolated from *B. subtilis* wild-type 168 and the *sigB* mutant ML6 before the stress-inducing treatment (co), 10 min after heat shock (h), treatment with 4% ethanol (e) or 4% NaCl (s) and 30 min after glucose starvation (g).

yhdF, *yhxC* and *yhxD* of *B. subtilis*, a glucose dehydrogenase in embryos of *Hordeum vulgare* (Alexander *et al.*, 1994) as well as glucose dehydrogenase isoenzymes of *B. megaterium* (Nagao *et al.*, 1992; Table 2). The presence of a short-chain dehydrogenase/reductase family signature (Joernvall *et al.*, 1995) suggests that YdaD might be involved in the generation of NAD(P)H.

The *ydaD* gene was cotranscribed with *ydaE* as a bicistronic mRNA. The protein coding sequence of the *ydaE* gene showed only a weak similarity to an unknown protein of *Morganella morganii* (accession no. L34345; Gallo & Mortlock, 1991). A stress-inducible transcript of about 1.6 kb was detected only in wild-type cells using *ydaD*- and *ydaE*-specific antisense RNA probes (Fig. 3a, b). The strongest induction occurred after cells were exposed to ethanol and salt. A weaker but significant induction also occurred after heat shock. A putative termination structure is located downstream of *ydaE*. Two additional less-induced transcripts of about

2.8 kb and 3.3 kb were detected after ethanol stress, presumably caused by a read-through of the potential termination structure (see Figs 3a, b and 7a, b).

As shown in Fig. 3(c), a stress-inducible transcript was detected starting 30 nt upstream of the translational-start codon of *ydaD* in the wild-type but not in the *sigB* mutant. The identified RNA polymerase-recognition sequence of *ydaD* (GTTTAT-N₁₄-AGGTAC) differed in two positions (underlined) from the consensus sequence of σ^B -dependent promoters (Haldenwang, 1995; Hecker *et al.*, 1996).

(iii) A weak resemblance of YdaP was observed to bacterial pyruvate oxidases like PoxB of *E. coli* (Chang *et al.*, 1994) and SpxB of *Streptococcus pneumoniae* (Spellerberg *et al.*, 1996; Table 2). These enzymes catalyse the conversion of pyruvate into acetate or acetyl phosphate, respectively. *E. coli* PoxB, which is under the control of σ^S , seems to be responsible for the generation of C₂ units from pyruvate during the transition state

Fig. 1. Protein synthesis pattern of *B. subtilis* 168 during exponential growth (a) and 10 min after treatment with 4% ethanol (b). Bacteria were grown in synthetic medium and labelled with L-[³⁵S]methionine as described in Methods. For a better orientation a few σ^B -dependent GSPs as well as the proteins ClpC, ClpP, AhpC, AhpF, SodA and TrxA are labelled (see Antelmann *et al.*, 1997a). The newly identified stress proteins are marked with boxes.

*The number of identical residues over the total length of the alignment is shown in parentheses.

Fig. 4. Analysis of the *ydaP* gene. See Fig. 2 legend for details.

Fig. 5. Analysis of the *yhdN* gene. See Fig. 2 legend for details.

pression of *ydaP* is σ^B dependent (Fig. 4a, b). Primer-extension experiments revealed that the upstream sequence of *ydaP* (GTTTAA-N₁₄-TGCTAT) resembles the consensus sequence of σ^B -dependent promoters, deviating only in one position in the -10 region (Fig. 4c). If *ydaP* really encodes a pyruvate oxidase it might be the fifth example of a gene (besides *dps*, *katE*, *opuE* and *osmC*) homologous to σ^S -dependent genes of *E. coli* that is under the control of σ^B in *B. subtilis*.

(iv) YhdN showed a relatively weak similarity to a number of potassium ion-channel proteins in eukaryotic and prokaryotic organisms. The *yhdN* gene was transcribed in a σ^B -dependent manner under stressful conditions. In addition to the *yhdN*-specific 1.2 kb transcript, a larger ethanol-inducible transcript of 1.8 kb (probably containing *yhdN* and *yhdO*) was detected (Fig. 5a, b). Both transcripts were missing in the *sigB* mutant. The promoter region of *yhdN* (GTTTAA-N₁₄-GGGAAA) was in accordance with the consensus sequence of σ^B -dependent promoters.

Gsp16u was identified as YocK with relatively weak similarity to the *Chlamydia trachomatis* and *E. coli*

DnaK suppressor protein DksA (Stephens *et al.*, 1998; Kang & Craig, 1990; Table 2). Using a *yocK*-specific RNA probe, a monocistronic transcript of 0.5 kb was detected after stress in the wild-type but not in the *sigB* mutant (Fig. 6a, b). The putative σ^B -dependent promoter (GTTTGA-N₁₃-GGGAAA) deviates only in one position from the consensus sequence.

(v) In this section, new σ^B -dependent proteins with still unknown function are described that show similarity to hypothetical proteins in the databases. The *ydaE* gene is cotranscribed with *ydaD* as the promoter-proximal gene and was described in section (ii). The amino acid sequence of YdaG displayed no similarities to any known proteins in databases. Several stress-inducible *ydaG*-specific transcripts were found (0.47, 0.6 and 0.9 kb; Fig. 7a, b). The *ydaG* gene was additionally part of the 2.8 kb and 3.3 kb transcripts which were detected with *ydaD*- and *ydaE*-specific RNA probes (Fig. 3a, b). A heat-inducible 1.6 kb transcript was detected in the *sigB* mutant that probably comprises the genes *ydaF*, *ydaG* and the 0.5 kb region between *ydaG* and *ydaH* (Fig. 7a, b). The coding region of *ydaG* is preceded by a

Fig. 6. Analysis of the *yocK* gene. Details are as for Fig. 2, except g_{30} = 30 min, g_{60} = 60 min after entry into stationary phase due to glucose starvation.

Fig. 7. Analysis of the *ydaG* gene. Details are as for Fig. 2.

σ^B -dependent promoter consensus sequence (GTTTAA-N₁₄-TGGAAA) and the 5' end of the *ydaG* message was detected by primer extension experiments (Fig. 7c).

Gsp18 was identified as YfkM showing similarities to hypothetical proteins of *E. coli* and *B. subtilis* (Table 2). The search for σ^B promoters revealed the existence of such a sequence upstream of *yfkM* (GTTTAT-N₁₃-GGGTAG).

DISCUSSION

B. subtilis cells induce a large number of GSPs in response to different stressful conditions. The majority of these GSPs are induced by heat, osmotic, acid or

ethanol stress as well as by energy depletion and they belong to the σ^B -dependent general stress regulon. A few years ago hardly anything was known about the physiological role of this regulon. By systematic studies relying either on a transposon-mutagenesis approach (Boylan *et al.*, 1991) or on proteomic studies (Völker *et al.*, 1994; Hecker *et al.*, 1996; Antelmann *et al.*, 1997a; Bernhardt *et al.*, 1997) first data on the function of the GSPs were presented. The σ^B -dependent general stress response might fulfil a similar physiological role as the σ^S -dependent response in *E. coli*, thus providing a non-specific, multiple and general stress resistance to non-growing cells (Gaidenko & Price, 1998; Hecker & Völker, 1998). The protection of DNA, proteins and membranes against oxidative damage might represent

an essential component within the complex stress response (Engelmann & Hecker, 1996; Antelmann *et al.*, 1997b). To obtain additional experimental evidence for the suggested physiological role of the σ^B regulon, new GSPs were identified by N-terminal sequencing of proteins which were no longer stress-inducible in a *sigB* mutant. The putative functions of these new proteins provide additional information on the physiological role of the entire regulon in *B. subtilis*.

(i) New σ^B -dependent genes were found that may provide new information on the physiological role of the regulon. The finding that *bmrU* as well as the *bmrUR* operon are under the control of σ^B indicates that the σ^B -mediated multiple stress resistance might be extended to the interaction with, and the elimination of, multidrug compounds in *B. subtilis*. The *bmrUR* operon encodes proteins that provide resistance against multidrug compounds. Bmr causes the efflux of various toxic compounds such as ethidium bromide, rhodamine 6G, tetraphenylphosphonium and fluoroquinolone antibiotics out of cells (Ahmed *et al.*, 1994). The most downstream gene (*bmrR*) encodes BmrR which is able to bind these substances and to act as a positive transcriptional regulator for *bmr* transcription (Ahmed *et al.*, 1994; Markham *et al.*, 1996). A specific *bmr* induction is mediated by BmrR in response to toxic and antibiotic compounds, while non-specific *bmr* induction depends on σ^B under stressful conditions.

Prokaryotic as well as eukaryotic organisms possess multidrug-resistance efflux transporters (for a review see Lewis, 1994) whose expression is induced by various structurally divergent compounds such as antibiotics, inhibitors and other toxic substances. In *Candida albicans* and *Saccharomyces cerevisiae*, expression of the multidrug ABC transporters *CDR1* (Krishnamurthy *et al.*, 1998), *PDR5* and *SNQ2* (Hirata *et al.*, 1994; Miyahara *et al.*, 1996) is stimulated by drugs, human steroid hormones and heat shock. The *E. coli* *acrAB* operon, encoding a drug-efflux pump, is induced by treatment with salt and ethanol, after transition into stationary phase (Ma *et al.*, 1995), and also by drugs like acriflavine (Ma *et al.*, 1993). These data underline the involvement of multidrug resistance in general stress resistance.

(ii) Our idea that σ^B -dependent GSPs might be involved in the maintenance of the intracellular redox balance under stress as well as in protection against oxidative damage (Hecker & Völker, 1998) might be strengthened by the identification of the σ^B -dependent proteins YcdF and YdaD. On the basis of their similarities to several NAD(P)-dependent dehydrogenases it might be possible that YcdF and YdaD are involved in the generation of NAD(P)H, which could play an important role as a reduction equivalent in the oxidative stress response (Farr & Kogoma, 1991; Dowds, 1994; Scharf *et al.*, 1998). The maintenance of a constant oxidoreduction state within the cell requires the reduction of oxidized biological molecules which may need NAD(P)H. The σ^B -dependent thioredoxin TrxA appears to be an

essential protein in *B. subtilis* (Scharf *et al.*, 1998) and might reduce oxidized thiol groups of proteins, leading to the restoration of active proteins. Oxidized TrxA becomes reduced by the thioredoxin reductase TrxB using NAD(P)H as a reduction equivalent. Another σ^B -dependent protein, NadE, participates in NAD synthesis (Antelmann *et al.*, 1997c). Therefore, we suggest that a sufficient level of NAD(P)H seems to be a prerequisite for the cell to face oxidative stress. However, further experiments are necessary to provide evidence for this assumption.

(iii) New members of the σ^B regulon whose orthologous genes are under the σ^S -control in *E. coli* strengthen the suggestion that both regulons fulfil a similar physiological role. Besides *katE* and *dps* (Engelmann *et al.*, 1995; Antelmann *et al.*, 1997b), the orthologues of the σ^S -dependent genes *proP* and *osmC* in *E. coli* are under σ^B control in *B. subtilis* (von Blohn *et al.*, 1997; Völker *et al.*, 1998). In this paper, another gene that is regulated by σ^B is described, namely *ydaP* of *B. subtilis*. Whether YdaP is indeed an acetyl phosphate-producing enzyme remains to be investigated.

(iv) The induction of σ^B -dependent genes in response to high osmolarity led to the suggestion that some GSPs participate in osmoprotection. This assumption was supported by the finding that the proline uptake transporter OpuE is regulated by high osmolarity and σ^B (von Blohn *et al.*, 1997; Spiegelhalter & Bremer, 1998). It is worth mentioning that YhdN, which is also σ^B -dependently expressed, resembles potassium ion-channel proteins. Potassium ion uptake is one of the first responses of cells after a sudden osmotic upshift (Csonka & Epstein, 1996). Furthermore, YocK, which shows weak similarity to the *E. coli* DksA protein, was identified as a σ^B -dependent protein. *E. coli* DksA was shown to suppress the temperature-sensitive and filamentous phenotype of *dnaK* mutations (Kang & Craig, 1990).

(v) The entire sequence of the *B. subtilis* genome shows that approximately 42% of the genes encode proteins with still unknown functions (Kunst *et al.*, 1997). It is a big challenge for future research to obtain information on the physiological role of these unknown proteins. The allocation of these proteins to regulons with known functions is a strategy to obtain initial information on the function of these unknown proteins within the framework of the regulon. New proteins with still unknown functions were identified that belong to the σ^B -stress/starvation regulon. It is highly probable that these proteins are also somehow involved in non-specific and prospective protection against stress. However, this is only a preliminary and general prediction. To define the function of each single new member of this regulon, a detailed analysis of the stress response of corresponding mutants must be conducted.

ACKNOWLEDGEMENTS

We would like to thank R. Schmid (Universität Osnabrück) for the protein sequencing and U. Völker (Philipps-Universität

Marburg, Laboratorium für Mikrobiologie und Max-Planck-Institut für Terrestrische Mikrobiologie, Marburg) for critical discussions and his support at the beginning of the experimental work. Many thanks to R. Lee (University of Wales, Cardiff) and T. Pummi (National Public Health Institute, Helsinki) for the helpful reading of the manuscript and S. Schade for the preparation of the photographs. This work was supported by grants from the Deutsche Forschungsgemeinschaft, the BMBF and the Fonds der Chemischen Industrie to M.H.

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Received 31 July 1998; revised 14 November 1998; accepted 3 December 1998.