Analysis of the induction of general stress proteins of *Bacillus subtilis*

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In *Bacillus subtilis* stress proteins are induced in response to different environmental conditions such as heat shock, salt stress, glucose and oxygen limitation or oxidative stress. These stress proteins have been previously grouped into general stress proteins (Gsps) and heat-specific stress proteins (Hsps). In this investigation the N-terminal sequences of 13 stress proteins of *B. subtilis* were determined. The quantification of the mRNA and the analysis of the protein synthesis pattern support the initial hypothesis that the chaperones DnaK and GroEL are Hsps in *B. subtilis*. In contrast, the recently described proteins GsiB, Ctc and RsbW belong to a class of Gsps that are induced by various stresses including heat shock. The main part of the Gsps described in this study failed to be induced in the *sigB* deletion mutant ML6 in response to heat shock. However, all the five Hsps were induced in this mutant in response to heat shock. These data indicate that SigB plays a crucial role in the induction of general stress genes, but is dispensable for the induction of Hsps.

Keywords: *Bacillus subtilis*, stress proteins, heat shock induction, *sigB*

INTRODUCTION

Bacteria spend most of their life time in a starving or non-growing state because they are frequently faced with different adverse environmental conditions most typical in nature (Roszak & Colwell, 1987; Morita, 1988). The capability of surviving under natural growth-restricting conditions needs special strategies that are of fundamental significance for microbial life in natural ecosystems. Therefore a very complex adaptational network has evolved in bacteria to keep cell viability under stress and ensure persistence and regrowth in the environment.

For *Bacillus subtilis*, living in the upper layers of soil, oxygen starvation may be especially important because *B. subtilis* is not able to generate energy solely by fermentation. Besides other common growth-limiting conditions like nutrient starvation, thermal or oxidative stress, salt stress should also be mentioned which may occur very frequently in soil.

The study of this adaptational network reflecting the dialogue between a *Bacillus* cell and its natural growth-limiting conditions is very important for the understanding of the physiology of Bacilli in general (Sonnenschein, 1989). An essential element of this network is the induction of stress genes. We found that the very strong induction of a typical set of proteins which we called general stress proteins (Gsps) is one of the first responses of *B. subtilis* to different natural growth-limiting conditions (Richter & Hecker, 1986; Hecker & Völker, 1990). Very effective stimuli of these stress proteins are glucose starvation or salt stress (Hecker *et al.*, 1988; Hecker & Völker, 1990).

We found that Gsps were also induced by heat shock (Hecker & Völker, 1990). However, some proteins were induced by heat but not by other growth-limiting conditions. In order to distinguish both groups of heat shock proteins the latter were called heat-specific stress proteins (Hsps; Richter & Hecker, 1986; Hecker & Völker, 1990).

Recently the genes encoding the well known chaperones...
GroEL and DnaK have been cloned and characterized by Schumann and co-workers (Schmidt et al., 1992; Wertzstein et al., 1992; Schön & Schumann, 1993) and Li & Wong (1992). Surprisingly the vegetative form of RNA polymerase Eσ32 appeared to be involved in the induction of both operons by heat shock. This is in contrast to Escherichia coli. The genes belonging to the heat stress regulon of E. coli are characterized by alternative promoters recognized by Eσ32 only. After heat stress the concentration of Eσ32 transiently increases more than tenfold and the heat shock proteins are induced (Neidhardt & VanBogelen, 1987). Furthermore the groEYL and the dnaK operons of B. subtilis exhibit an inverted repeat (TTAGCAGTC-NpGAAGTGCATA) which is conserved in the regulatory region upstream of genes coding for chaperones in Gram-positive bacteria, Cyanobacteria and even some Gram-negative bacteria (Nabenhauer & Bahl, 1992; Segal & Ron, 1993). It was suggested that this inverted repeat is somehow involved in the heat induction of these genes (Li & Wong, 1992; Schmidt et al., 1992). These data demonstrate that the mechanism of heat shock protein induction in B. subtilis may differ from that in E. coli (Schmidt et al., 1992).

Our main interest is the elucidation of the mechanisms which are responsible for the induction of the same set of stress proteins by diverse growth-limiting conditions. To obtain information on the structure of general stress genes, the N-terminal amino acid sequences of Gsps were determined. The changes in the level of mRNA of several genes, the N-terminal amino acid sequences of Gsps were determined. Furthermore the induction of stress proteins in response to heat shock was analysed in a sigB deletion mutant. The data presented in this paper point to a crucial role of the alternative σ factor SigB in the induction of Gsps in B. subtilis.

### METHODS

#### Bacterial strains and culture conditions.

*Escherichia coli* DH5α was used for the transformation and propagation of the plasmids used in this investigation. DH5α was routinely grown in a complex medium. *Bacillus subtilis* strains IS58 (trpC2 lys; Smith et al., 1980) and ML6 (trpC2 sigB::ΔHindIII–EcoRV::cat; Igo et al., 1987) were cultivated at 37 °C in a synthetic medium described previously (Stülke et al., 1993). Different stress conditions were provoked according to the following procedures. Heat stress; cells were cultivated to a density of 5 × 10^8 cells ml⁻¹ and transferred to 48 °C: salt stress; at a density of 5 × 10^8 cells ml⁻¹, NaCl was added to a final concentration of 4% (w/v); glucose limitation; bacteria were grown in a synthetic medium with limiting amounts of glucose (0.05%); oxygen limitation; at a density of 5 × 10^8 cells ml⁻¹, the culture was transferred to a closed centrifuge tube and the incubation was continued at 37 °C without shaking: oxidative stress; during exponential growth, bacteria were exposed to 0.005% H_2O_2. The time of the shift was set to zero. The samples were taken during exponential growth immediately prior to the shift or at the time indicated in the relevant figure legends.

#### Pulse labelling and two-dimensional protein electrophoresis.

Bacteria were labelled at the time indicated in the relevant figure legends with 1.5 μCi (55.5 kBq) L-[³⁵S]methionine ml⁻¹ [specific activity, 1000 Ci mmol⁻¹ (37 TBq)] for 5 min. The disruption of the cells by ultrasonic treatment and the two-dimensional electrophoresis of the protein extracts were carried out as described previously (Hecker et al., 1988).

### Microsequencing of proteins.

For the analysis of the N-terminal protein sequence the proteins were cut from the two-dimensional protein gels and collected. The gel pieces of 30–50 gels were applied onto a preparative polyacrylamide gel, separated and transferred onto a PVDF-membrane by electroblootting. The proteins were sequenced on an Applied Biosystems A473a protein sequencer.

#### Analysis of transcription.

Total RNA of *B. subtilis* IS58 was isolated by the acid phenol method described by Majumdar et al. (1991) with the following modifications. Bacteria (10^9 cells) were collected by centrifugation at 4 °C and washed with chilled killing buffer (20 mM Tris/HCl, pH 7.5; 5 mM MgCl₂; 20 mM NaNO₃). Cells were resuspended in 1 ml buffer (25%, w/v, sucrose; 50 mM Tris/HCl; pH 8.0; 0.25 mM EDTA) containing 1 mg lysozyme ml⁻¹, incubated for 5 min on ice and the protoplasts were harvested by centrifugation. After resuspension in 600 μl lysis buffer (3 mM EDTA, 200 mM NaCl, 0.5% SDS), lysis of protoplasts was achieved by incubation for 5 min at 95 °C. The samples were extracted three times with acid phenol/chloroform/isooamylalcohol (25:24:1, by vol.), twice with chloroform/isooamylalcohol (24:1, w/v) and once with diethylether. After ethanol precipitation the RNA was dissolved in A. bidest. Decreasing amounts of total RNA were transferred onto a positively charged nylon membrane by slot-blotting, hybridized with digoxigenin-labelled probes and detected according to the manufacturer's instructions (Boehringer Mannheim). The filters were exposed to Fuji RX films and the hybridization signals were quantified with a Personal Densitometer from Molecular Dynamics. The induction ratios were calculated by dividing the volumes of the signals obtained from RNA of stressed cells by the volume of the signals obtained from RNA isolated from the controls (37 °C, exponential growth). The hybridizations specific for groEL and dnaK were conducted with digoxigenin-labelled RNA synthesized in vitro with T7 and SP6 RNA polymerase from linearized plasmids pSEG247 and pSED377. These plasmids are derived from pSPT18 (Boehringer Mannheim) and contain a 860 bp EcoRI–HindIII fragment encoding GroEL or a 840 bp BglII fragment encoding a part of DnaK (Fig. 1). The plasmids were linearized at the end of the insert distal to the T7 or SP6 promoter with HindIII or EcoRI, respectively. Digoxigenin-labelled RNA was synthesized from the coding (SP6 polymerase) and the non-coding strand (T7 polymerase). The RNA synthesized in vitro from the coding strand was used as a negative control for the hybridization and did not yield any specific hybridization signal (data not shown).

The mRNAs specific for sigB, ribW, sigB, ribX, ctc and xynA were quantified with the help of digoxigenin-labelled probes which were prepared from PCR fragments corresponding to the coding regions of sigB, ribW, sigB, ribX, ctc and xynA (Fig. 1). These DNA–RNA hybridizations were performed according to the manufacturer's instructions (Boehringer Mannheim). The identity of the PCR products was confirmed by dye-labeled sequencing according to Sanger (1977) with both PCR primers as primers for the sequencing. The following primers were used for the amplification:

\[
\text{sigB} \quad S1 \quad 5'-\text{CGAGGAATTTGTCAAAAAC} 3', \text{operon} \\
S2 \quad 5'-\text{ATAAATACGCGTCACTCCTC} 3' \\
\]
RESULTS

Identification of general stress proteins on two-dimensional O'Farrell gels

Recently the chaperones GroEL and DnaK, and the ClpP protease of *B. subtilis* have been identified on two-dimensional protein gels by N-terminal microsequencing (Miller *et al.*, 1991; Völker *et al.*, 1992; Fig. 2). In this work the N-terminal sequences of several other stress proteins were determined (Figs 2 and 3, Table 1). Among these stress proteins we also found GrpE, a protein belonging to the *dnaK* operon (Wetzstein *et al.*, 1992). A comparison of the N-terminal sequences with databases allowed the identification of three further stress proteins of *B. subtilis* on two-dimensional polyacrylamide gels (Figs 2 and 3), which are encoded by the genes *gsiB* (Mueller *et al.*, 1992), *rsbW* (Benson & Haldenwang, 1992; Boylan *et al.*, 1992) and *ctc* (Igo & Losick, 1986; Ray *et al.*, 1988). It is interesting to note that the promoters of both *ctc* and *rsbW* are recognized by SigB of *B. subtilis* (Igo & Losick, 1986; Kalman *et al.*, 1990).

The N-terminal sequences of the general stress proteins Gsp9, Gsp17o, Gsp17m, Gsp20a, Gsp22 and Gsp26 did not show any significant sequence homology with proteins in the database (Fig. 2).

**Induction of stress proteins by heat shock and other stresses**

Several stimuli like heat shock, salt stress, oxidative stress or nutrient starvation induced Gsps. Proteins induced mainly by heat stress were classified as Hsps (Hecker & Völker, 1990). According to our first two-dimensional protein studies, the well-known chaperones GroEL and DnaK might be Hsps (Hecker *et al.*, 1988; Völker *et al.*, 1992). In Table 1 recent results on the induction of stress proteins by various stimuli are summarized. The Gsps Ctc, RsbW, ClpP, GsiB, Gsp9, Gsp13, Gsp14, Gsp17o, Gsp17m, Gsp20a and Gsp26 were induced by heat shock, salt stress, oxidative stress, glucose limitation and oxygen limitation. All these different environmental stimuli induced the transition of the cells from a growing state into a non-growing state. This induction of stress proteins was one of the earliest responses of the cell to growth limitation. All these different environmental stimuli allowed the identification of three further stress proteins we also found GrpE, a protein belonging to the *dnaK* operon (Fig. 1).

**Fig. 1.** Schematic presentation of the probes used for the hybridization. The location of the genes and the direction of transcription are indicated by labelled arrows. The thicker black lines represent the regions which were used as probes for the hybridization. Restriction enzymes: B, BglII; C, CiaI; D, Dral; E, EcoRI; H, HindIII; P, PvuII. The positions of the PCR-primers are marked S1, S2, C1, C2, G1, G2, X1 and X2.

For the analysis of the *gsiB* mRNA, a 480 bp *Dral–PvuII* fragment was used as probe. The probe specific for *ctc* was prepared by labelling the entire PCR fragment with digoxigenin by the random primer method. The hybridization experiments for *rsbW*, *sigB* and *rsbX* were performed with a 560 bp *Dral* fragment, a 880 bp *Dral–ClaI* fragment and a 1200 bp *ClaI* fragment, respectively. The quantification of the *xynA* mRNA was carried out with the entire PCR fragment labelled with digoxigenin (Fig. 1).

**Fig. 2.** N-terminal sequences of stress proteins of *Bacillus subtilis*. The sequences were determined as described in Methods.

<table>
<thead>
<tr>
<th>Protein spot</th>
<th>Class</th>
<th>Sequence</th>
<th>Protein</th>
</tr>
</thead>
<tbody>
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<td>Heat</td>
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<td>GroEL</td>
</tr>
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<td>Heat</td>
<td>SEVIGID</td>
<td>DnAEL</td>
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<td>GrpE</td>
</tr>
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<td>General</td>
<td>SDRIVVYSTD</td>
<td></td>
</tr>
<tr>
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<td>General</td>
<td>ALPTAKYTAR_GGAAHITED_D</td>
<td></td>
</tr>
<tr>
<td>Gsp17m</td>
<td>General</td>
<td>NIVYVYETNY_DNIKAEYNER_L</td>
<td></td>
</tr>
<tr>
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<td>General</td>
<td>AQK_VYFPQ_MAIXQXEL</td>
<td></td>
</tr>
<tr>
<td>Gsp22</td>
<td>General</td>
<td>MLNIGEVYVLP_FERK</td>
<td></td>
</tr>
<tr>
<td>Gsp26</td>
<td>General</td>
<td>MNQIOEKXVL</td>
<td></td>
</tr>
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<td>MLULIPIVIQ_THRAHERATDI_YBRL</td>
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<td>General</td>
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<tr>
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<td>MKNADYIEM_KVDVQ</td>
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Fig. 3(a, b). For legend see p. 746.
General stress proteins in *Bacillus subtilis*

**Fig. 3(c, d).** For legend see p. 746.
Table 1. Scheme of induction of stress proteins in *B. subtilis* by different stimuli

The bacteria were exposed to various stimuli as described in Methods and the relative synthesis rate before and after the imposition of stress was compared. −, Repression; 0, no change in synthesis rate; + to ++++, increasing level of induction. The data represent the mean induction ratios of at least three independent experiments. Fig. 3 shows the autoradiograms of one of these experiments. ND, Not determined.

<table>
<thead>
<tr>
<th>Protein</th>
<th>Heat shock</th>
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<th>Oxidative stress</th>
<th>Glucose limitation</th>
<th>Oxygen limitation</th>
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<tr>
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Restricting conditions, which was already detectable 2 min after stress imposition (not shown).

In contrast, the classification of Hsp1, Hsp2, GroEL and DnaK as Hsps could be confirmed. GrpE was strongly induced by heat, but the other stimuli did not induce GrpE (Fig. 3, Table 1). Therefore GrpE, which is encoded by the same operon as DnaK, belongs to the group of Hsps too.

Analysis of the transcription of stress genes by slot-blot hybridization

To verify the pattern of induction of stress proteins obtained by two-dimensional gel electrophoresis, the level of mRNA of the stress genes *dnaK, groEL, gsiB, ctc, ribW, sigB* and *ribX* was determined before and after the exposure to different stresses. The specific mRNA was detected by slot-blot analysis of total RNA prepared either from exponentially growing cells or from bacteria harvested at different times after stress. A serial dilution of RNA was spotted onto nylon membranes and hybridized with specific digoxigenin-labelled RNA or DNA probes as described in Methods (Fig. 4a).

The amount of mRNA specific for *rsbW, sigB* and *ribX*, encoded by the *sigB* operon, *gsiB* and *ctc* increased transiently after the bacteria were exposed to heat shock, oxygen limitation or salt stress (Fig. 4b). This increase of the mRNA level after stress agreed with the data of the protein synthesis pattern. GsiB, RsbW and Ctc were synthesized with an increased rate after the imposition of heat shock, oxygen limitation and salt stress (Fig. 3 and Table 1).

The amount of *dnaK* and *groEL* mRNA increased transiently by a factor of 5–8 after a heat shock as...
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Fig. 4(a, b). For legend see p. 748.
described previously (Schmidt et al., 1992; Wetzstein et al., 1992). Salt stress and the exhaustion of glucose did not trigger an increase in the mRNA level of these operons (Fig. 4c) and only the groEL operon was induced by oxygen limitation. Overall this induction profile agreed with the protein synthesis pattern determined in response to the same stimuli. However, there was no significant increase in the protein synthesis rate of GroEL after oxygen limitation.

For all the genes and the conditions tested the induction was transient reaching a maximum between 6 and 12 min after the bacteria were exposed to the stimuli. The changes in the mRNA level observed after stress were specific because the amount of dnaK mRNA did not rise during the first 30 min in response to any condition analysed in this study (data not shown). XynA encodes the xylanase of B. subtilis which is constitutively expressed during growth and was used as a control (Lindner et al., 1994).

**Induction of stress proteins by heat shock in a sigB mutant**

The alternative σ factor SigB of B. subtilis is involved in the heat shock response (Benson & Haldenwang 1993) and seems to be responsible for the induction of a large stationary-phase regulon too (Boylan et al., 1993). The promoters of the general stress genes ctc and rsbW are recognized by a RNA-polynerase-containing SigB. Therefore the question arose whether SigB has a fundamental role in the induction of the stress proteins in B. subtilis. The synthesis of stress proteins in the sigB mutant ML6 during heat shock was analysed by two-dimensional protein electrophoresis (Fig. 5). The mutation in sigB did not influence the induction of Hsps by heat shock. However, the majority of the Gsps described in this study failed to be induced by heat shock in the mutant strain ML6, among them Ctc, RsbW and GsiB. The induction of the Gsps ClpP and Gsp12 was not influenced by the sigB mutation.

**DISCUSSION**

**Classification of stress proteins**

In B. subtilis there are several proteins which are induced by heat stress as well as by other stimuli. As in E. coli it is possible to classify specific subsets of stress proteins according to their induction pattern (VanBogelen et al., 1987; Matin, 1991; Hengge-Aronis, 1993). A detailed analysis of the induction of proteins as well as of changes in mRNA levels after the imposition of different stimuli enabled us to confirm our suggestion that chaperone genes and general stress genes belong to different gene groups characterized by different induction patterns (see Richter & Hecker, 1986; Hecker et al., 1988; Hecker & Völker, 1990). However, our transcriptional studies consider the mRNA levels only. Additional investigations have to establish whether the increased mRNA levels after stress are due to increased transcriptional rates or increased mRNA stabilities. In preliminary experiments, however, we did not find any hint of an increase in the stability of mRNA of groEL caused by heat stress.

The proteins GroEL, GrpE, DnaK as well as Hsp1 and Hsp2 belong to the group of Hsps induced by heat stress. Salt stress, glucose starvation and oxidative stress did not induce these proteins. The discrepancy between the increase in the mRNA level and the unchanged protein synthesis rate of GroEL after the limitation of oxygen is currently being investigated.
Fig. 5. Synthesis of stress proteins in the *Bacillus subtilis* *sigB* mutant strain ML6 during exponential growth and after the imposition of heat shock. Bacteria were pulse-labelled with L-[³⁵S]methionine before (37 °C; a) and 10 min after the transfer to 48 °C (b) for 5 min. The squares mark stress proteins, the induction of which requires the alternative σ factor SigB. Hst, Proteins induced by heat or starvation.
The other group of stress proteins was induced by various stimuli. It is tempting to speculate that these Gsps may provide an unspecific protection of the cell under non-growing or starving conditions, regardless of the environmental factor which induced the non-growing state.

In contrast, stress-specific proteins are presumably induced by a specific stimulus only, because they exert a specific protection against the extracellular signal and not against any other. This specific response could be achieved by destroying the signal (e.g. superoxide dismutase destroys superoxide radicals) or by a protective response against the results of the stressors action. According to this view Hsps may provide a specific protection against heat damage. It is well known that during heat stress, malfolded or even denatured proteins are produced more frequently which can be renatured by an increased amount of chaperone proteins (Craig & Gross, 1991).

After salt stress we found neither a dramatic increase in the synthesis rate of DnaK and GroEL nor a strong increase in the mRNA level of both transcripts. This is a surprising result because the levels of DnaK or GroEL have been shown to be elevated in E. coli after starvation or salt stress (Jenkins et al., 1991; Meury & Kohiyama, 1991).

Furthermore, it should be mentioned that stress proteins specific for salt stress, oxidative stress and nutrient starvation have been detected as well, but these stress-specific proteins were not investigated in this study (Richter & Hecker, 1986; Hecker et al., 1988).

Stress proteins with similar unspecific induction kinetics like the proteins we called Gsps in B. subtilis (Richter & Hecker, 1986) have already been described in literature. In Cyanobacteria, Bhagwat & Apte (1989) distinguished between salt-stress- or heat-stress-specific and unspecific stress proteins. In E. coli the PEX proteins are required for general protection of the cell against stress (Matin, 1991; Hengge-Aronis, 1993). A universal stress protein was investigated by Nyström & Neidhardt (1992) who analysed a σ^70-dependent gene which was induced by quite different stresses.

**Mechanisms of induction of stress proteins**

According to the induction pattern described in this investigation, general stress genes might require regulatory proteins for heat shock induction other than the typical heat shock genes. The chaperone genes dnaK and groEL, which exhibited a rather heat-specific induction contain an inverted repeat immediately after the startpoint of transcription that seems to be involved in the heat induction mechanism, e.g. by binding a repressor protein or by forming a transcriptional termination signal (Li & Wong, 1992; Schmidt et al., 1992; Wetzstein et al., 1992). The proteins Hsp1 and Hsp2 displayed similar induction kinetics and might contain the same inverted repeat in front of the coding gene. In contrast, the Gsps are characterized by an entirely different induction profile and the general stress genes analysed so far do not possess this conserved inverted repeat upstream of the coding region. Moreover, our data support the assumption that the general stress genes do not form a unique gene group, but probably consist of more than one regulon.

Sonenshein and coworkers characterized the glucose-starvation-induced protein GsiB, which was produced at higher rates after the cells have been starved for either glucose or phosphate, or after the reduction of the GTP-pool (Mueller et al., 1992). We could demonstrate that gsiB is strongly induced in response to heat shock, oxidative stress or salt stress as well. Sonenshein and coworkers have been unable to demonstrate any *in vitro* transcription of gsiB by purified Eσ^70-RNA polymerase (Mueller et al., 1992).

The classification of the SigB-dependent Ctc protein as well as of RsbW as Gsps strongly suggests that SigB is involved in the induction of a group of Gsps. RsbW is the second cistron in the sigB operon which is transcribed by Eσ^70 (Kalman et al., 1990; Boylan et al., 1992). It is well known that σ^70-dependent genes are active in the stationary phase (Boylan et al., 1992). However, the physiological role of the SigB has been a matter of speculation for more than 10 years (Haldenwang & Losick, 1979; Duncan et al., 1987). Very recently, Benson & Haldenwang (1993) reported that the sigB operon as well as ctc are induced by heat shock. Price and co-workers have initiated a genetic approach in order to define the stationary phase regulon which is controlled by SigB (Boylan et al., 1993). The results of our investigation suggest that the increase in the amount of SigB or the activation of SigB may be key events in the induction of Gsps. This view is supported by our findings that sigB operon-specific mRNA levels are transiently increased after heat stress as well as after salt stress and oxygen limitation and by the fact that the major group of the Gsps described in this paper failed to be induced in response to heat shock in a SigB mutant. During heat shock and salt stress the transcription of the sigB operon initiated at the same site as during stationary phase (unpublished results; Benson & Haldenwang, 1993; Kalman et al., 1990). The heat shock induction of the protein GsiB is dependent on the presence of SigB and the transcription of gsiB initiated at the same site as described by Mueller et al. for glucose starvation (1992) when the bacteria were exposed to heat shock or salt stress (data not shown). The sequence just upstream of this site of initiation (Mueller et al., 1992) closely resembles promoters which are effectively transcribed by Eσ^70 (Varon et al., 1993). It is tempting to assume that SigB is the σ factor responsible for the induction of the major group of general genes by heat stress as well as by other stimuli.

However, some of the general stress genes seem to be subjected to separate control mechanisms because the proteins encoded were induced to the same level by heat shock in the SigB mutant (e.g. ClpP and Gsp12).

**Concluding remarks**

The induction pattern of Hsps, including GroEL and DnaK and Gsps by heat shock and other stimuli has been
described. With the help of the N-terminal sequences, the proteins GsiB, Ctc and RsbW could be identified as Gsps. Furthermore the alternative $\sigma$ factor SigB seems to be responsible for the induction of the majority of general stress genes in B. subtilis.

The mechanism of the induction of SigB-dependent genes by either heat or physiological stress will be the subject of future investigations. Furthermore, we will analyse the structure and function of SigB-dependent general stress genes in more detail to get information on the role of Gsps for the survival of adverse environmental conditions.

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