The Bacillus subtilis clpC operon encodes DNA repair and competence proteins

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ClpC of Bacillus subtilis, controlling competence gene expression and survival under stress conditions, is encoded by the fourth gene of a six-gene operon. The product of orf1 contains a potential helix-turn-helix motif, but shows no significant similarities with known protein sequences. The second and third genes encode proteins with similarities to zinc-finger proteins (orf2) and arginine kinases (orf3), respectively. The product of orf5 contains a zinc-finger motif and an ATP-binding domain, and is highly similar to the product of the Escherichia coli sms gene. A strain bearing a disruption of orf5 showed increased sensitivity to the alkylating agent methyl methanesulfonate. Furthermore, this mutant strain displayed decreased capacity for genetic recombination as measured by transformation experiments. The last open reading frame, orf6, encodes a protein with limited similarity in its C-terminal part to the B. subtilis comEA gene product and to the UvrC DNA repair excinuclease. Inactivation of orf5 resulted in strongly diminished transformation with all types of DNA. Mutations affecting either orf5 or orf6 resulted in strains with decreased resistance to UV-irradiation in the stationary phase, indicating that these proteins play a role in the development of a non-specific stationary-phase resistance to UV-irradiation. Moreover, these results suggest an involvement of both proteins in transformation and presumably in DNA repair.

Keywords: stress proteins, Bacillus subtilis, ClpC, DNA repair, competence

INTRODUCTION

For soil bacteria such as Bacillus subtilis the ability to survive unfavourable environmental conditions is crucial. In order to respond to stressful situations or starvation, the cell has developed an adaptive network including stress responses and post-exponential-phase phenomena such as the development of genetic competence, motility, synthesis of degradative enzymes and finally sporulation. These processes are controlled by a complex and incompletely understood regulatory network accompanied by interactions and overlaps between the components (Msadek et al., 1995).

Genetic competence of B. subtilis, the ability to take up exogenous DNA molecules, is controlled by many regulatory genes, such as spo0A, comP, comA, degU, comS, mecA, clpC/mecB and comK. These genes are required for appropriate expression of the late competence genes, encoding DNA-binding and -uptake enzymes (Dubnau et al., 1994). Moreover, the SOS system is also part of the competence response, and is subject to a dual regulation: a damage-inducible pathway and a competence-dependent induction (Dubnau, 1991; Cheo et al., 1993).

Synthesis of bacterial stress proteins is induced by a wide variety of factors including heat shock, ethanol stress, pH and osmolarity changes, UV-irradiation, hydrogen peroxide, DNA-damaging agents and starvation (Bukau, 1993; Hecker et al., 1996). ClpC, a member of the stress-response-related Clp-ATPase family, is a pleiotropic regulator involved in stress tolerance and cell division (Krüger et al., 1994). The ClpC protein was also identified as MecB, controlling competence and synthesis of degradative enzymes (Msadek et al., 1994). Therefore, ClpC may provide links between the competence pathway and the stress response. Originally, the mecA and mecB mutations were isolated as allowing competence gene expression in complex media (Dubnau & Roggiani, 1990). Interaction of MecB with MecA

Abbreviation: MMS, methyl methanesulfonate.
negatively regulates the synthesis of ComK, a transcrip-
tional activator required for the expression of late
complement genes (Kong & Dubnau, 1994; Msadek
et al., 1994). Expression of the clpC gene, encoding
the general stress protein GSP12, was previously shown to
be induced by various stress conditions (Krüger et al.,
1994). The nucleotide sequence of a 180 kb region
containing clpC was reported as part of the B. subtilis
genome sequencing project, suggesting that clpC is the
gene of an operon containing six open reading
frames (Ogasawara et al., 1994). We recently showed
that the six genes are cotranscribed as an operon,
preceded by two promoters. One resembles promoters
recognized by the vegetative RNA polymerase Eσ+. The
other promoter was shown to be stress-inducible and
dependent upon σB, the general stress σ-factor in B.
subtilis (Haldenwang, 1995). However, in a sigB mutant,
the σB-like promoter became inducible, completely
compensating for σB deficiency (Krüger et al., 1996).
Even in the absence of σB this promoter switch ensures
a similar rate of protein synthesis as in the wild-type.
In addition, we found conditions which exclusively induce
the vegetative σB promoter such as amino acid limitation
or hydrogen peroxide (Krüger et al., 1996).

Two genes of the clpC operon, orf3 and orf5, were
cloned previously and designated orfX and orfY
(Msadek et al., 1994). Orf5 shares significant similarities
with the Escherichia coli sms gene product (sensitivity to
methyl methanesulfonate; Neuwald et al., 1993). POTen-
tial functions of the orf5 and orf6 gene products
coded by this operon were investigated in this study.

METHODS

Bacterial strains, plasmids and culture conditions. Bacterial
strains are listed in Table 1. E. coli strains DH5α and RR1
were routinely grown in LB medium and used as hosts for
DNA manipulation. B. subtilis was cultivated with vigorous
aeration at 37 °C in LB medium or a previously described
synthetic medium (Stülke et al., 1993). Media were supple-
mented with the following antibiotics when necessary:
ampicillin (100 μg ml⁻¹), chloramphenicol (5 μg ml⁻¹),
spectinomycin (100 μg ml⁻¹), or erythromycin (1 μg ml⁻¹) plus
lincomycin (25 μg ml⁻¹). The SPAC promoter (Pspac)
(Yansura & Henner, 1984) was induced by treatment with
1 mM IPTG.

Sensitivity to methyl methanesulfonate (MMS) was tested
according to Neuwald et al. (1992) by growing the cells in LB
into the exponential growth phase and plating them in soft
agar onto LB plates. A paper disc with 10 μl MMS was placed
in the centre of the plate, giving a final concentration of 0.05
MMS. To investigate survival after UV-irradiation, appro-
priate dilutions of cells from the exponential phase, the
transition phase or the stationary phase were plated onto LB
agar and exposed to UV light for 10 s (8 W cm⁻², 312 nm,
Vilbert Lourmat transilluminator) and incubated overnight at
37 °C. The distance between the agar surface and the UV
source was 1 cm.

General methods. Plasmid isolation, restriction enzyme analy-
sis, transformation of E. coli, ligation of DNA fragments
and filling in the recessed 3' termini using the Klengon fragment
of DNA polymerase I were performed according to standard
protocols (Sambrook et al., 1989). Recombinant plasmids
were sequenced by the dideoxy chain-termination method of
Sanger et al. (1977). DNA fragments were amplified by PCR as
described earlier (Krüger et al., 1994). Some oligonucleotides
used for PCR included mismatches allowing creation of EcoRI
and BamHI restriction sites. Chromosomal DNA from B.
subtilis was isolated as described by Msadek et al. (1991).
Transformation of B. subtilis with plasmid or chromosomal
DNA was carried out using a two-step protocol (Hoch, 1991).

Construction of plasmids for generating orf5 and orf6 mutant
strains. pMEC32 was constructed by deleting a 1826 bp
HindIII fragment from pMEC28 (Msadek et al., 1994),
removing the last 111 codons of orf3 and the first 497 codons
of clpC. A 1238 bp SmaI fragment carrying the spectinomycin
resistance gene from Staphylococcus aureus (Murphy, 1985)
was then cloned into the unique PstI site within pMEC32, to give pMEC39. Plasmid pMEC39 was linearized by EcoRI digestion and transformed into B.
subtilis, selecting for spectinomycin resistance, indicating a
double crossover event (strain BEK9). To exclude transform-
ants carrying the mutation as a Campbell-type integra-
tion, spectinomycin-resistant transformants were tested for
erythromycin sensitivity.

The orf5 disruption in strain BEK9 was verified by PCR using
cromosomal DNA of the transformant as a template. Strain
BE01 was constructed by cloning the 531 bp ScaI-BamHI
orf5 fragment of pMEC28 into pDH88 (Henner, 1990) and
transforming IS58 with the resulting plasmid pDSM. In strain
BE01 orf5 is disrupted by a single crossover recombination
event allowing reinitiation of transcription by Pspac down-
stream of orf5.

pMEC60 was constructed by cloning a 200 bp BamHI PCR
fragment internal to orf6 using oligonucleotides TM-116 (5'-
GGGAGTCCGATGCCGGATGCCACAATTTC-3') and
TM-117 (5'-GGGAGATCCAGTTTGTGATGCTTGG-
TTC-3') into plasmid pHT181 (Lereclus & Arantes, 1993).
When integrated by a Campbell-type event, this internal
fragment gave rise to two truncated copies of orf6 (strain
BEK60). All Campbell-type disruptions were verified by
Southern-blot analysis using digoxigenin-labelled PCR frag-
ments as probes according to the manufacturer’s recom-
mandation (Boehringer Mannheim).

RESULTS

Predicted protein sequence analysis

The predicted amino acid sequences of the five other
open reading frames cotranscribed with clpC (Fig. 1)
were compared with the GenBank database using the
BLAST (Altschul et al., 1990) network service at the
National Center for Biotechnology Information, with
the default parameter values provided. Only matches
with a probability lower than e⁻⁷ were considered
significant, except in cases noted below.

A chromosomal locus of the facultative pathogen
Listeria monocytogenes was identified recently, dis-
playing a genetic organization similar to that of the clpC
locus in B. subtilis (Rouquette et al., 1996). Strong
similarities were noted between Orf1, Orf2, Orf3, ClpC
and Orf5 of B. subtilis and L. monocytogenes, with
amino acid sequence identities of 64%, 29%, 50%,
73% and 70%, respectively (Rouquette et al., 1996).
Table 1. Strains and plasmids used in this study

<table>
<thead>
<tr>
<th>Strain/plasmid</th>
<th>Relevant genotype</th>
<th>Source/reference</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>E. coli</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>RR1</td>
<td>F- mcrB mrr bsdS20 (F m) ara-14 proA2 lacY1 leu galK2 rpsL20 (Sm') xyl5 mtl-1 supE44</td>
<td>Bolivar et al. (1977)</td>
</tr>
<tr>
<td>DH5a</td>
<td>F’. g80d lacZA M15 Δ(lacZYA–argF)U169 deoR recA1 endA1 hisD17 (R m) supE44 thi-1 gyrA96</td>
<td>Hanahan (1985)</td>
</tr>
<tr>
<td><strong>B. subtilis</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>IS58</td>
<td>trpC2 lys-3</td>
<td>Smith et al. (1980)</td>
</tr>
<tr>
<td>BEO1</td>
<td>trpC2 lys-3 sms::pDH88</td>
<td>pDSMS → IS58</td>
</tr>
<tr>
<td>BEK9</td>
<td>trpC2 lys-3 sms::spc</td>
<td>pMEC29 → IS58</td>
</tr>
<tr>
<td>BEK56</td>
<td>trpC2 lys-3 amyE::(orf1-'lacZ aphA3)</td>
<td>Krüger et al. (1996)</td>
</tr>
<tr>
<td>BEK60</td>
<td>trpC2 lys-3 orf6::pHT181</td>
<td>pMEC60 → IS58</td>
</tr>
<tr>
<td><strong>Plasmids</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>pAC7</td>
<td>Ap' Km' lacZ</td>
<td>Krüger et al. (1996)</td>
</tr>
<tr>
<td>pDH88</td>
<td>Ap' Cm' Pspac</td>
<td>Henner (1990)</td>
</tr>
<tr>
<td>pHT181</td>
<td>Ap' Em'</td>
<td>Lereclus &amp; Arantes (1993)</td>
</tr>
<tr>
<td>pH331</td>
<td>Neo' Cm'</td>
<td>Gärnter et al. (1988)</td>
</tr>
<tr>
<td>pSPEC</td>
<td>Ap' Spc'</td>
<td>P. Trieu-Cuot, unpublished</td>
</tr>
<tr>
<td>pDSMS</td>
<td>pDH88 derivative with a 531 bp fragment of orf5</td>
<td>This work</td>
</tr>
<tr>
<td>pMEC28</td>
<td>Ap' Em' E. coli/B. subtilis shuttle vector containing part of orf3, clpC and part of orf5</td>
<td>This work</td>
</tr>
<tr>
<td>pMEC32</td>
<td>pMEC28 derivative constructed by deleting a 1826 bp HindIII fragment</td>
<td>This work</td>
</tr>
<tr>
<td>pMEC39</td>
<td>pMEC32 derivative carrying the spectinomycin resistance gene in the unique PstI site</td>
<td>This work</td>
</tr>
<tr>
<td>pMEC60</td>
<td>pHT181 derivative containing a 200 bp BamHI PCR fragment internal to orf6</td>
<td>This work</td>
</tr>
</tbody>
</table>

However, an equivalent of orf6 of *B. subtilis* is not present in the reported *L. monocytogenes* nucleotide sequence (GenBank accession number U40604), where instead orf5 is followed by an open reading frame whose product shows similarities with that of a gene lying downstream from orf6 in *B. subtilis*. Except for ClpC, no functional role has been established for the products of orf1, 2, 3 and 5 in *L. monocytogenes* (Rouquette et al., 1996).

The product of orf1 (154 aa) of the *B. subtilis clpC* operon displays no significant similarities to proteins in the database. However, a predicted helix–turn–helix DNA-binding motif was detected in Orf1 using the weight matrix of Dodd & Egan (1990), beginning at amino acid 29 (RSEIADKFQCVPSQINYVIN, SD score 4.12. HTH1 probability > 90%). This suggests that Orf1 may have a regulatory role. Interestingly, this potential helix–turn–helix domain is highly conserved in the orf1 product of *L. monocytogenes*.

Similarities between Orf2 (185 aa) of *B. subtilis* and proteins in the database were noted with a large family of eukaryotic regulatory proteins, characterized by a zinc-finger nucleic-acid-binding domain (Klug & Rhodes, 1987; Vallee et al., 1991). Indeed, Orf2 contains two potential Cys2-Cys2 zinc-finger motifs, at positions Cys2-Cys2 and Cys8-Cys8, to Cys2-Cys2 and Cys8-Cys8. orf3 encodes a putative protein (363 aa) with a domain that is highly conserved among ATP:guanidino phosphotransferases such as arginine kinases (e.g. *Homerus vulgaris*; Dumas & Camonis, 1993) or creatine kinases (e.g. *Schistosoma mansoni*; Stein et al., 1990). This domain extends from amino acids 119 to 251 of Orf3, presenting 35% sequence identity on average with members of this family of kinases.

As previously reported (Msadek et al., 1994; Ogasawara et al., 1994), the deduced amino acid sequence of orf5 (458 aa) shows 46% identity with that of the *E. coli* *sms* gene, whose absence confers sensitivity to MMS.
Fig. 2. Alignment of the B. subtilis Orf5 amino acid sequence with that of the E. coli sms gene product (Neuwald et al., 1992). Identical residues are shaded. ATP-binding A and B motifs (Walker et al., 1982) are boxed, and a potential zinc-finger nucleic-acid-binding domain is underlined.

(Neuwald et al., 1992) (Fig. 2). The orf5 and sms gene products both contain conserved ATP-binding A and B motifs (Walker et al., 1982) as well as a potential zinc-finger nucleic-acid-binding domain (positions C_{10}-C_{13} to C_{24}-C_{27}, Fig. 2). The sms gene appears to be highly conserved among prokaryotes and is also present in Salmonella typhimurium, Haemophilus influenzae and Synechocystis sp. Sms proteins also show similarities with RecA, in the vicinity of the ATP-binding site, and the carboxy-terminal region shows similarities with Lon proteases (Neuwald et al., 1992).

Although the potential orf6 gene product (360 aa, formerly orf360; Ogasawara et al., 1994) shows no strong sequence similarities with proteins in the database, we noted some slight similarities with several proteins that interact with nucleic acids, such as DNA ligase of Thermus thermophilus and ATP-dependent RNA helicase of Methanococcus jannaschii. Indeed, a short carboxy-terminal region of Orf6 (amino acids 300-358) shows approximately 30% sequence identity with a corresponding stretch in these proteins as well as the E. coli UvrC DNA repair excinuclease (Sancar et al., 1984) and ComEA of B. subtilis (Inamine & Dubnau, 1995).

Hydropathy profiles of the products of the six genes in the clpC operon, determined by the method of Kyte & Doolittle (1982), suggested that these proteins are all cytoplasmic since no significantly hydrophobic potential transmembrane regions were noted (cutoff value: 2.6).

The sms phenotype of an orf5 mutant

As indicated above, the deduced product of the fifth open reading frame shows about 50% amino acid sequence identity with the E. coli sms gene product. The orf5 gene was disrupted, generating mutant BEK9.

To examine the possibility that orf5 is indeed an sms homologue in B. subtilis, sensitivity to the alkylating agent MMS was tested using a paper disc method as described by Neuwald et al. (1990). Several independent experiments were performed by plating exponentially growing cultures of the BEK9 (orf5) and BEK60 (orf6) mutants in parallel with the corresponding wild-type strain IS58 on agar plates with a paper disc containing MMS (see Methods). Diameters of clear inhibition zones were measured. For a given strain, the diameters varied by less than ±2 mm. Treatment with MMS (0.05%) resulted in average inhibition zones that were reproducibly larger in diameter for the mutant strain BEK9 (46 mm) than for the wild-type strain (40 mm), which is comparable to results obtained with the E. coli sms mutant. Strain BEK60 gave results nearly identical to the wild-type. These results suggest that a mutation in orf5 leads to increased sensitivity of the mutant cells to
MMS, as described for E. coli. We therefore propose that orf5 be designated srns.

Survival of orf5 and orf6 mutants after UV-irradiation

Stationary-phase cells of E. coli are more resistant to UV-irradiation than exponentially growing cells, in a σ8-dependent manner (for a review see Hennge-Aronis, 1993). Functional similarities between the σ8 regulon in E. coli and the σB regulon in B. subtilis have been described for the katE gene (Engelmann et al., 1995; Engelmann & Hecker, 1996). Furthermore, the clpC operon is partly σB-dependent.

To investigate the UV survival response of mutants affected in the clpC operon, we first examined the survival of B. subtilis wild-type IS58 cells following exposure to UV-irradiation at different growth phases in glucose limitation medium (GLM) and under amino acid limitation (competence medium; CM). Samples were taken during exponential growth at an OD560 of 0.4, in the transition phase (OD560 0.8 for GLM; OD560 1.0 for CM) or 1 h after entry into stationary phase (OD560 1.0 for GLM; OD560 1.3 for CM). Only amino-acid-starved cells showed an approximately five-fold increased resistance to UV-irradiation; glucose-starved cells showed no increase in resistance (data not shown). Therefore, competence medium was used for further experiments.

Since Orf5 and Orf6 have similarities with DNA-repair enzymes, we investigated mutants in both genes for survival after UV-irradiation. To avoid polar effects of the insertional mutation within orf5 another mutant was constructed using plasmid pDSMS, allowing re-initiation of transcription by Pspac. This mutant, BEO1, also shows the srns phenotype. In all experiments IPTG was added to the culture of strain BEO1 to a final concentration of 1 mM. Several independent UV-irradiation experiments were performed, resulting in approximately 1% survival for the wild-type during the exponential growth phase. However, the number of viable wild-type cells increased to 7% and 5% during the transition and stationary phases, respectively. Setting survival of the wild-type to 100%, only 15–20% of the mutants BEO1 and BEK60 cells survived this procedure in transition and stationary phase, whereas in the exponential growth phase the sensitivity of the mutants was less pronounced (Table 2). These results suggest the involvement of Orf5 as well as Orf6 in the development of a non-specific stationary-phase resistance to UV-irradiation of amino-acid-starved B. subtilis cells. Results obtained with the orf5 mutant BEK9 after UV-irradiation were similar to those with the BEO1 mutant, indicating that polar effects do not play a role (data not shown).

Orf5 and Orf6 are involved in genetic competence

We had previously noted that a clpC deletion mutant showed a decrease in transformability compared to a mutant with a point mutation in clpC (T. Msadek, unpublished). Given the slight similarities of the carboxy-terminal domain of Orf6 with ComEA, which is essential for competence development in B. subtilis, we investigated the possible role of Orf6 in competence. Since the SOS response and the development of genetic competence overlap (Cheo et al., 1993), it was interesting to test whether competence is also influenced in an orf5 mutant. Transformation experiments were carried out by preparing competent cells of strains BEO1, BEK60 and the corresponding wild-type strain IS58 in parallel. Cells were transformed with the replicative plasmid pWH331 (Gärtner et al., 1988), with pAC7, a plasmid which cannot replicate in B. subtilis but integrates via a single crossover event in the amyE locus, or with chromosomal DNA of strain BEK56 requiring a double crossover event (Krüger et al., 1996), with selection for the corresponding antibiotic resistance. Transformation

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**Table 2. Survival after UV-irradiation of B. subtilis wild-type strain in comparison to orf5 and orf6 mutants in different growth phases in competence medium**

<table>
<thead>
<tr>
<th>Growth phase</th>
<th>Wild-type IS58</th>
<th>orf5 mutant BEO1†</th>
<th>orf6 mutant BEK60</th>
</tr>
</thead>
<tbody>
<tr>
<td>Exponential (OD₅₆₀ 0.4)</td>
<td>1.3 × 10⁻² (±0.32 × 10⁻²)</td>
<td>0.9 × 10⁻² [69.2%] (±0.28 × 10⁻²)</td>
<td>1.1 × 10⁻² [84.6%] (±0.31 × 10⁻²)</td>
</tr>
<tr>
<td>Transition (OD₅₆₀ 1.0)</td>
<td>7.1 × 10⁻² (±0.5 × 10⁻²)</td>
<td>1.4 × 10⁻² [14.6%] (±0.5 × 10⁻²)</td>
<td>1.25 × 10⁻² [17.6%] (±0.6 × 10⁻²)</td>
</tr>
<tr>
<td>Stationary (OD₅₆₀ 1.3)</td>
<td>4.95 × 10⁻² (±0.15 × 10⁻²)</td>
<td>0.9 × 10⁻² [18.2%] (±0.5 × 10⁻²)</td>
<td>1.02 × 10⁻² [20.6%] (±0.5 × 10⁻²)</td>
</tr>
</tbody>
</table>

*Survival after UV-irradiation is indicated as the ratio of colony-forming units with and without UV treatment. Means of five independent experiments are shown, with standard deviations indicated in parentheses. For the orf5 and orf6 mutants, percentage survival in comparison to the wild-type is shown in square brackets.

†Cells of strain BEO1 were cultivated with the addition of 1 mM IPTG to induce Pspac.

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Table 3. Transformability of orf5 and orf6 mutants in comparison to the wild-type

<table>
<thead>
<tr>
<th>DNA type</th>
<th>Frequency of transformation (%)*</th>
<th>Wild-type IS58</th>
<th>orf5 mutant BE01†</th>
<th>orf6 mutant BEK60</th>
</tr>
</thead>
<tbody>
<tr>
<td>Replicating plasmid (pWH331)</td>
<td>0.81</td>
<td>0.58 (1.4x)</td>
<td>0.18 x 10^(-1) (45x)</td>
<td></td>
</tr>
<tr>
<td>Non-replicating plasmid (pAC7)</td>
<td>0.12</td>
<td>0.52 x 10^(-2) (23x)</td>
<td>0.32 x 10^(-2) (37x)</td>
<td></td>
</tr>
<tr>
<td>single crossover</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Chromosomal (BEK56)</td>
<td>0.18</td>
<td>0.49 x 10^(-2) (37x)</td>
<td>0.51 x 10^(-2) (35x)</td>
<td></td>
</tr>
</tbody>
</table>

* Frequency of transformation is indicated as (kanamycin-resistant transformants/viable cells) x 100. The means of four independent experiments are shown; standard deviations were less than 10% of the mean. The numbers in parentheses indicate x-fold decrease of transformation frequency of the mutant strains in comparison to the wild-type.

† Cells of strain BE01 were cultivated with the addition of 1 mM IPTG to induce Pspac.

experiments are summarized in Table 3. Transformation with a replicative plasmid was not strongly influenced in the sms mutant BE01 in comparison to the wild-type. However, transformation rates dropped significantly with chromosomal DNA, requiring recombination events. The orf6 disruption led to a strong decrease of competence in every case, indicating that orf6 might be a novel competence gene. We therefore suggest that orf6 be renamed comY.

DISCUSSION

To study the functions of the other five unknown gene products encoded by the clpC operon, database analysis was performed and phenotypes of selected mutants were investigated. The high probability of a helix-turn-helix motif within the amino acid sequence of Orf1 suggests a possible regulatory role for this protein. Preliminary results of our group indeed show that Orf1 may act as a repressor of the clpC operon expression under non-stressed conditions (E. Krüger & M. Hecker, unpublished).

For the fifth gene, orf5, whose product is similar to that of the E. coli sms gene (Neuwald et al., 1992), similar phenotypes were observed for the mutant, leading to mild sensitivity to the alkylating agent MMS during exponential growth phase. Hence, the gene product may participate in repair of DNA damage by alkylation or DNA repair in general. This assumption is underlined by the increased sensitivity of sms (orf5) mutants to UV-irradiation. The increased sensitivity was also observed for mutants in orf6, suggesting that Orf6, which shows some weak similarities to nuclease genes, is also involved in DNA repair. Recently, we found that a σB- and a presumably σA-dependent promoter directs stress-induced transcription of the clpC operon. Expression from the σA-like promoter alone could be induced in response to stress conditions that do not induce σB, such as hydrogen peroxide treatment or amino acid starvation (Krüger et al., 1996). Increased stationary-phase resistance to UV-irradiation was shown for amino-acid-starved but not for glucose-starved cells, indicating that this phenomenon is mediated by a σB-independent mechanism. Our results suggest that Sms (Orf5) as well as ComY (Orf6) are involved in this mechanism, emphasizing an important function for σA-dependent induction of the clpC operon. However, the function of both Sms (Orf5) and ComY (Orf6) might only be important for the development of a non-specific stationary-phase resistance to UV-irradiation in addition to the constitutive excision and recombination repair systems involving the recA and the uvrA loci (Friedman & Yasbin, 1983).

Disruption of the sixth open reading frame, orf6, resulted in a significant decrease in competence for DNA transformation. Although there is no indication of the membrane association of Orf6, according to the hydropathy calculation method of Kyte & Doolittle (1982), one cannot exclude a role for this protein in DNA uptake, as observed for mutants in the comEA locus (Albano et al., 1987; Hahn et al., 1993; Inamine & Dubnau, 1995). An sms (orf5) mutation led to decreased transformability with DNA requiring homologous recombination. In view of the results discussed above and the inducibility of the clpC operon by DNA-damage-inducing treatment with hydrogen peroxide (Krüger et al., 1996), an involvement of the sms and comY (orf5 and orf6) gene products in either DNA-damage repair or competence, or both, could explain the phenotypes of these mutants. The link between the two mechanisms is an apparent overlap in the regulatory mode of the competence-dependent induction of the SOS system and the regulation of the competence regulon itself (Cheo et al., 1993). However, previous data suggest that mutations in the regulatory genes of the competence pathway do not significantly affect vegetative-phase expression of a clpC-lacZ fusion (Msidek et al., 1994). Furthermore, the consensus sequence found upstream of DNA-damage-inducible genes (GAAC-N_4-GTTC; Cheo et al., 1993) is not present in the clpC operon. This suggests that DNA-damage induction of the clpC operon may involve a different mechanism from that of the din regulon. Protection of DNA or proteins against stress damage is essential for bacteria to keep existing cellular structures in a functional state. B. subtilis has developed efficient systems for detoxification and repair of DNA.
under stress conditions. Interestingly, these include $\sigma^B$-dependent and $\sigma^H$-independent general stress proteins. The H$_2$O$_2$-inducible genes katA and mrkA, the din genes, the alkyl hydroperoxide reductase operon abpC/abpF and the superoxide dismutase gene sod encode enzymes responsible for a specific oxidative stress resistance (Antelmann et al., 1996; Bol & Yasbin, 1994; Cheo et al., 1993; Chen et al., 1995). However, genes of the $\sigma^B$ regulon of B. subtilis seem to encode proteins that provide a non-specific protection of the cell against oxidative stress during starvation (Engelmann & Hecker, 1996). Our results suggest that SmS and ComY (Orf5 and Orf6) might belong to such non-specific systems. In this context, an overlap between the $\sigma^B$-dependent and $\sigma^H$-independent general stress responses, the SOS response and competence for DNA transformation seems plausible.

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REFERENCES


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