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# Mutant analysis of interaction of the *Bacillus subtilis* transcription regulator AbrB with the antibiotic biosynthesis gene *tycA*

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The AbrB protein of *B. subtilis* represses the transcription of various postexponentially expressed genes, such as the antibiotic biosynthesis gene *tycA*. Recently, we have shown that AbrB binds to the *tycA* promoter region at two A+T-rich sites; the 'promoter site' (−60 to −35) and the 'leader site' (+169 to +231). In this study we demonstrate that a $P_{tyc}-lacZ$ fusion missing the leader region is constitutively expressed in wild-type *B. subtilis* cells and in *B. subtilis* cells carrying *spoOA* or *abrB* mutations. We also show that substitution mutations within the recently reported potential helix–turn–helix DNA binding motif of AbrB did not affect its specific DNA binding ability.

*Bacillus subtilis; tycA* Expression; AbrB mutants

## 1. INTRODUCTION

In *B. subtilis*, SpoOA protein is required for the activation of a wide variety of postexponentially expressed genes including those associated with the process of endospore formation and the production of secondary metabolites [1]. Mutations in the suppressor locus *abrB* can overcome the dependence on SpoOA for the transcription of the *aprE* gene (encoding subtilisin [2]), the *tycA* gene (encoding the tyrocidine synthetase 1 [3]) and the *spoOE* and *spoVG* genes [4,5], which are associated with the sporulation process. The *abrB* gene encodes a 10.5 kDa DNA binding protein, whose expression is under SpoOA control [6–8]. SpoOA is a part of a signal transducing system that senses the nutritional environment [9–12]. The current model assumes that AbrB negatively affects transcription of the genes under its control by directly binding to their promoter regions [4,13]. At the transition state between exponential and stationary phases of growth the activated SpoOA protein represses *abrB* transcription, thereby relieving the repression of AbrB controlled genes such as *tycA*. *tycA* is constitutively expressed in *spoOA abrB* double mutants, indicating that AbrB may be the sole repressor of *tycA* transcription [3]. Recently we have shown by DNaseI footprinting, that AbrB binds to the *tycA* promoter region at two A+T-rich sites, within the promoter site and the leader sequence [14]. In the present study we examine the effect of deleting the 'leader AbrB binding site' on *tycA* transcription. In addition, we report on the substitution mutations into the previously reported [6] potential helix-turn-helix (HTH) DNA binding motif of AbrB protein and the analysis of their DNA binding activity by mobility shift experiments.

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## 2. MATERIALS AND METHODS

### 2.1. *Bacterial strains and plasmids*

The genotypes of bacterial strains and the structure of the plasmids used were described previously [3,13,14].

### 2.2. *Construction of the* $P_{tyc}$-lacZ *fusion and of SPβ transducing phages*

The *Eco*RI-*Hind*III fragment of pGEM3-381 [14] containing the *tycA* promoter region [15] without the 'leader AbrB binding site' was isolated and cloned into the *Hind*III-digested pZ delta 327 [3] after filling in the ends of both vector and insert by Klenow polymerase treatment. A plasmid of the desired construction (designated as pMZ12 delta) was identified by restriction analysis and verified by sequencing. DNA preparation, restriction endonuclease digestion, ligation and other standard methods used for DNA manipulation were as described by Maniatis [16]. For DNA sequencing the method of Sanger [17] was applied. In order to construct SPβ transducing phages carrying the *tyc-lacZ* fusion, the SPβ lysogen ZB 307A was transformed [18] with pMZ12 delta and plated on DSM medium plates selecting for blue chloramphenicol-resistant colonies. Isolation and purification of SPβ transducing phage from *B. subtilis* lysogenes were as previously described [5,19].

### 2.3. *β-Galactosidase assays*

Cells containing the *tyc-lacZ* fusion were grown in DSM medium to an optical density at 595 nm of 0.2. Then samples (1 ml) were taken at regular time intervals and were assayed for β-galactosidase specific activity as previously described [20,21].

### 2.4. *Site directed mutagenesis of the* abrB *gene*

The 1.4 kb *Pst*I-*Hind*III fragment of pP12 delta P containing the *abrB* coding sequence (M. Gocht, Diplomarbeit, TU Berlin, 1988)

was cloned into *PstI* + *HindIII* digested pBluescriptII KS⁻ using TG 1 cells as host and single-stranded DNA was isolated. For site-directed mutagenesis we used the oligonucleotide-directed mutagenesis system version 2 of Amersham as recommended by the supplier. The sequences of mutagenic oligonucleotides were as follows: Helix 1, 5′-GTTATCATCAGGAACTTCACC-C′; Turn, 5′-TTTACCGCCTG-TAAGTTTAAG-3′; Helix 2, 5′-TTCTTTACTAGGAACCAAT-TT-3′. The oligonucleotide 5′-TTATTTAAGGTTTTGAAGCTG-3′ was used as a primer for sequence analysis of *abrB* mutants. All oligonucleotides were purchased from TIB MOLBIOL (Berlin).

2.5. *Purification of AbrB mutants and DNA binding experiments*

Purification of AbrB and analysis of DNA binding activity of the AbrB mutants by gel retardation experiments were performed as previously described [13,14].

## 3. RESULTS AND DISCUSSION

### 3.1. *A deletion mutation within the* tycA *promoter region has the same phenotype as an* abrB *mutation*

In *B. subtilis* AbrB protein appears to be the sole repressor of *tycA* transcription, as this gene is postexponentially expressed in wild-type cells and constitutively transcribed in *abrB* mutants [3]. We have shown recently [14] that AbrB directly interacts with the *tycA* promoter region at two sites, the 'promoter site' (−60 to −35) and the 'leader site' (+169 to +231). In this work we constructed a *PtycA-lacZ* transcriptional fusion that lacked the leader site (Fig. 1) and introduced it into various *B. subtilis* strains using SPβ-transducing phages. We studied the effect of the deletion mutation on *tycA* transcription by measuring the β-galactosidase-specific activity (Fig. 2). Our results show that two different kinds of mtuation (a *cis* mutation and a *trans* mutation: *abrB 703* [3]) gave rise to the same phenotype; a constitutive expression of the *tycA* gene. These results indicate that the remaining promoter site of AbrB binding is not sufficient to repress *tycA* expression during exponential growth. This is in keeping with the results of our previous DNaseI footprinting experiments [14] where AbrB protein bound to the promoter site only in the presence of the leader site on the same DNA fragment, indicating that both binding sites are necessary for effective binding in vitro. However,

```
AATATTTATC GTCAAAATAT AGCCGTACGC TTTCCTTTTT TATAGACAAG
TTGAAAAGGA AAAATGTTGC AGCGCCGAAA CGAGCCAATC ACTGGTTCAC
CAAAGGGAAA AACACGTGTG CCGCTTATTG ATTACGCAAG CGGGAGCAGG
TTTGCCAAGC AAAACTTGGA TTTACGTAAA AAGGTTGTAA AAAAACTTGT
                                        -35
TGAATTTTTT GCAAAATATC CCTATTTTTT AATCGACTTC CAATTTTTCT
    -10         +1
CTGCTATAAT GAGTTTCAGC GTCAGTAACC TAGTGCTTTC AGCCTGTCAG
CCGATCGGGG AACTTCCTGT GATTGTTTTC ATGCAAATCA GTTTTCCTTC
GTTCGCAGCG TAAGCAGGCG TATCCGGCAG CGGAATACCA GCACCCATTT

TCCGCTACAG CATGAGCAAA ATATGACTTT AGTATGAAGG GAATTTCCCA

GAAACAGGAA ATTTTCTTGT TGTTAAAATT ACCCAAATGA TGGAAAATGG
GAAATTGGAA TGGAACGTTG ACCTTGCCTG TCTCTTGTTG GCAACCATTT
CGTCACACTT CATGATAAGC AGAAGT
```

Fig. 1. Nucleotide sequence of the *tycA* promoter region. AbrB binding sites are indicated by lines. The region printed in bold letters was fused to the promoterless *lacZ* gene.

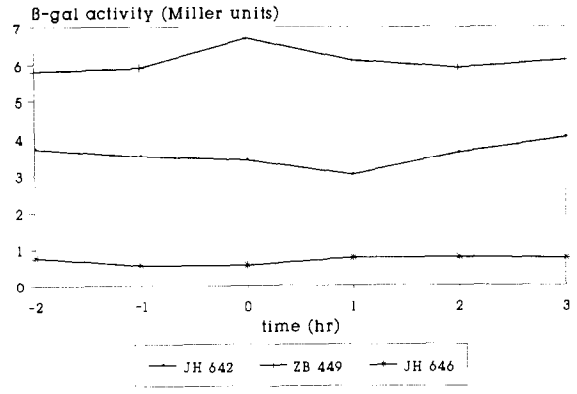


Fig. 2. β-galactosidase activity (Miller units) of the *Ptyc-lacZ* fusion in *B. subtilis*. JH 642 is wild type, JH 646 is a *spoOA* mutant and ZB 449 carries the *abrB 703* mutation. The time point 0 indicates the beginning of sporulation.

AbrB could bind to a DNA fragment containing an isolated leader site.

### 3.2. *DNA binding activity of AbrB mutants*

We planned to identify by site-directed mutagenesis the region of AbrB protein essential for DNA binding. As a beginning, we constructed AbrB proteins with substitution mutations within the previously published potential HTH motif [6] (Figs 3 and 4). The Pro residues introduced at position 61 and 74 are thought to be incompatible with the structure of α-helices and the Thr residue introduced at position 68 should interfere with the formation of the loop between the two α-helices (Fig. 3). The DNA binding activity of the mutant

```
          -35                 -10
AAAATGATTGACGATTATTGGAAACCTTGTTATGCTATGAAGGTAAGGATTTTGTCGAATAATGAC


GAAGAAAAATATAATTTAAACAAATAAGTATCTCTTGGGAGGAGAATGTTTATG AAA TCT ACT
1                                                        M   K   S   T

   GGT ATT GTA CGT AAA GTT GAT GAA TTA GGA CGT GTA GTT ATT CCT ATC
5  G   I   V   R   K   V   D   E   L   G   R   V   V   I   P   I

   GAA CTG CGT CGT ACT CTT GGA ATC GCA GAA AAA GAT GCT CTT GAA ATC
21 E   L   R   R   T   L   G   I   A   E   K   D   A   L   E   I

   TAT GTT GAT GAT GAA AAA ATC ATC CTT AAA AAA TAT AAA CCA AAC ATG
37 Y   V   D   D   E   K   I   I   L   K   K   Y   K   P   N   M

         A                       C                           A
   ACT TGC CAA GTA ACT GGT GAA GTT TCT GAT GAT AAC CTT AAA CTT GCA
53 T   C   Q   V   T   G   E   V   S   D   D   N   L   K   L   A
  AbrB4 Y                   Helix 1 P                       Turn T
                        C
   GGC GGT AAA TTG GTT CTT AGT AAA GAA GGC GCT GAG CAA ATC ATC AGC
69 G   G   K   L   V   L   S   K   E   G   A   E   Q   I   I   S
              Helix 2 P
   GAA ATC CAA AAC CAG CTT CAA AAC CTT AAA TAA TCATTTCTTGTACAAAAAA
85 E   I   Q   N   Q   L   Q   N   L   K  Ter                >>>>>>

CGTTCTTGTTATGACACAAGAACGTTTTTTTATTGCTTAATATGGTAAGC
>>>>>>>>>       <<<<<<<<<<<<<<<
```

Fig. 3. Nucleotide sequence of the *abrB* gene. The deduced amino acid sequence is shown below the nucleotide sequence. Also shown are point mutations used in this study. The potential terminator sequence is indicated by >><<

```
AbrB                    helix      turn      helix
54
C Q V T G E V S D D N L K _ _ L A G G K L V L S K E G A E Q I
  *   * * * * *   * * *   * * * *     * * *   * * *   *
      * *   *     * *     *   *       *       *
E L L R G E M S Q R E L K N E L G A G I A T I T R _ G S N S L
60
TrpR
```

Fig. 4. Comparison of the helix-turn-helix regions of the AbrB protein and the TrpR protein [6]. Similar residues are indicated with one star and identical residues with two stars.

proteins was analyzed by mobility shift experiments (Fig. 5). It appears that the putative HTH motif is not involved in specific DNA binding as none of the mutants is affected. Interestingly, AbrB4 protein with a $Cys^{54} \rightarrow Tyr^{54}$ substitution (Fig. 3) has lost its DNA binding activity entirely [7].

### 3.3. *Is AbrB a helix-turn-helix protein?*

We have searched for HTH motifs within the AbrB protein using the method of Dodd and Egan [22] which allows the systematic detection and evaluation of potential HTH motifs from protein sequences by calculating an'SD score'. The probability for a protein segment to adopt an HTH motif is high with an SD score of $\geqslant 2.5$. The SD score of the HTH motif of TrpR, a member of the master set of 91 known HTH-proteins is 2.5 [22]. The SD score of the published potential HTH motif of AbrB, after removal of the two gaps (see Fig. 4) is as low as $-3.9$, indicating a low probability for a HTH conformation. We did not find any other region within AbrB likely to adopt such a structure. In addition, AbrB does not contain any of the known DNA binding motifs [23] and we failed to identify homology to other known DNA binding proteins, using THE EMBL NETWORK FILE SERVER [24]. Therefore further mutants are needed to identify unequivocally the DNA binding motif of the AbrB protein.

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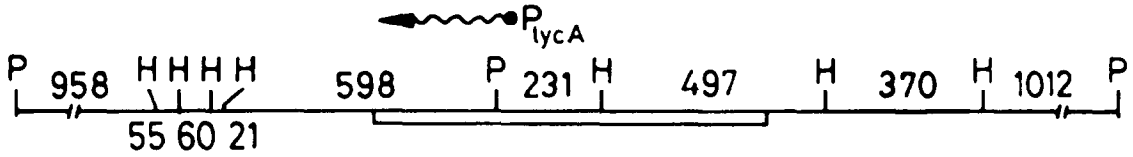


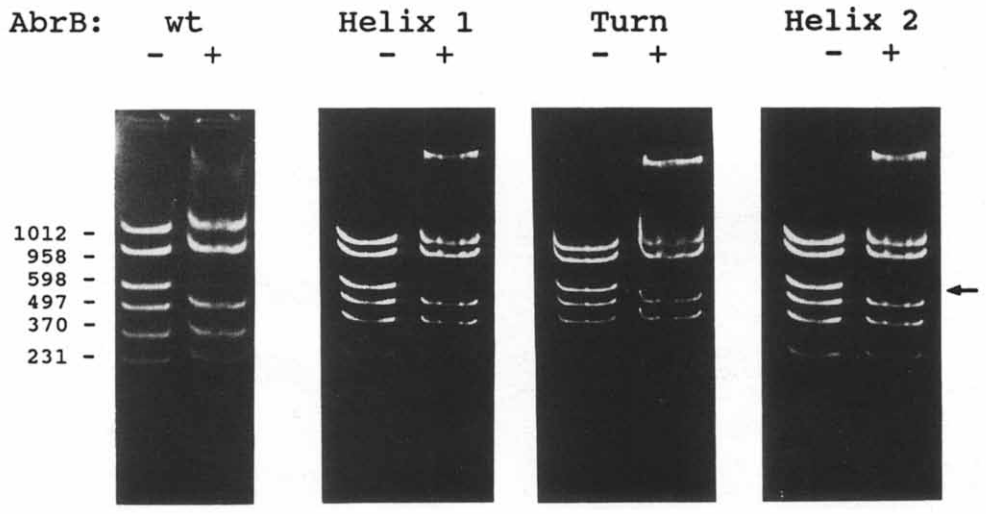


Fig. 5. (upper) Physical map of *PvuI–HaeII* digested pGEM3-38 containing the *tycA* promoter region. (lower) Mobility shift experiments using *PvuI* + *HaeII* digested pGEM3-38 and AbrB protein as indicated at concentrations of about 2 $\mu$M. The lengths of DNA fragments are given in base pairs. Arrow indicates the position of retarded bands.

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