Transcriptional analysis of the ylaABCD operon of Bacillus subtilis encoding a sigma factor of extracytoplasmic function family

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The ylaABCD operon of Bacillus subtilis contains four predicted ORFs in the order ylaA, ylaB, ylaC and ylaD, where ylaC is assumed to code for a sigma factor of the extracytoplasmic function (ECF) family. Predicted YlaD may function as the anti-YlaC factor as it has an oxidative stress sensing domain similar to that of the RsrA, which is the anti-sigma factor of SigR, an ECF sigma of Streptomyces coelicolor. Northern blot analysis of the ylaABCD operon revealed two transcriptional products resulting from a distal promoter upstream of ylaA and from an internal promoter located at the first codon of ylaC. Both transcription start sites were determined by primer extension and 5'-RACE PCR. The transcription from the distal promoter was initiated by over-expression of YlaC on a multi-copy plasmid and depended on YlaC. DNA sequences of the –35 and –10 regions were similar to those recognized by other ECF sigmas of B. subtilis. On the other hand the transcription from the internal promoter was induced by oxidative stress and depended on Spx, which is an oxidative stress responding factor interacting with the alpha subunit of RNA polymerase core enzyme. The latter transcription depended possibly on SigA. We could not detect translation of YlaC from this transcript. Experiments with ylaD-disruption or co-overexpression of ylaD with ylaC suggested that YlaD functions as the anti-YlaC factor. Although YlaD has an oxidative stress sensing domain, oxidative stress did not induce the whole ylaABCD operon.

Key words: B. subtilis, ECF sigma, ylaABCD operon

INTRODUCTION

Since the discovery of SigE of the Streptomyces coelicolor as a sigma factor which directs transcription of cell wall repairing genes (Lonetto et al., 1994), homologous sigma factors involved in extracytoplasmic function (ECF) have been studied in various bacteria and found to direct transcription of the relevant genes upon stresses such as denaturation of proteins, antibiotics, oxygen, alkali, heat, and cold (Bashyam et al., 2004; Helmann, 2002; Hughes et al., 1998; Missiakas et al., 1998; Ravio et al., 2001). Some bacteria possess more than ten paralogs of ECF sigma genes while there are many bacteria harboring no ECF sigma gene (Mittenhuber, 2002; Helmann, 2002). ECF sigma gene is usually associated with a gene encoding a membrane protein, which functions as an antisigma; however, the functions of most ECF sigmas and their antisigmas are not well studied. Best-studied is the ECF sigma SigE of Escherichia coli. Stress resulted in digestion of ECF sigma-bound anti-sigma factor RseA by DegS and YaeL proteases followed by release and activation of SigE (Ravio et al., 2001).

The whole genome sequencing of Bacillus subtilis revealed seven ORFs, sigM, sigV, sigW, sigX, sigY, sigZ, and ylaC; the amino acid sequences of the predicted protein products are similar to those of ECF family sigma factors (Kunst et al., 1997). They seem to constitute operons with downstream ORFs except for sigZ and ylaC. sigZ is monocistronic and ylaC is the third ORF of the presumed ylaABCD operon. The functions of SigV, SigZ and YlaC remain to be elucidated, while four sigmas, SigM, SigW, SigX, and SigY, have been studied well. They are required for stresses caused by salt, heat, ethanol, acid, cell wall antibiotics, superoxide, alkali, and nitrogen-starvation (Horsburg and Moir, 1999; Thackray and Moir, 2003; Cao et al., 2002B; Cao and Helmann, 2002; Cao et al., 2003; Huang et al., 1998; Tojo et al., 2001).
Gene disruption and in vivo protein interaction studies revealed that their anti-sigma factors are YhdL, RsiW(YbbM), RsiX, and YxIC, respectively (Helmann, 2002; Cao et al., 2003; Tojo et al., 2003; Yoshimura et al., 2004). Regulons of SigW and SigX are well elucidated (Cao et al., 2002A; Huang et al., 1998; Huang and Helmann, 1998; Peterson et al., 2001). Furthermore, regulon candidates for all seven ECF sigmas were extracted by microarray analysis, in which ECF sigma genes were over-expressed on a multi-copy plasmid (Asai et al., 2003).

As for SigV, YrhM seems to be the anti-SigV because there is direct interaction between SigV and YrhM in vivo and disruption of yrhM resulted in enhanced expression of the sigV-yrhM operon (Yoshimura et al., 2004). On the other hand, the ylaABCD operon of B. subtilis seems to consist of ylaA, ylaB, ylaC, and ylaD (Kunst et al., 1997). Presumably YlaC is similar to ECF sigma factors, and YlaD is similar to RsrA of ZAS family, a zinc-binding anti-sigma factor of the ECF sigma SigR of Streptomyces coelicolor, which has multiple cystein residues sensing the cytoplasmic thiol-disulphide status (Paget et al., 2001). YlaA, YlaB, and YlaD are considered to possess nine, one, and two transmembrane domains, respectively. Direct interaction was observed only between YlaB and YlaD by yeast two-hybrid analysis (Yoshimura et al., 2004). DNA microarray analysis found only three YlaC-regulon candidates rplJ, yjeA, and pshD, other than ylaA and ylaD of the ylaABCD operon (Asai et al., 2003). While YlaC possesses many orthologs, YlaA, YlaB and YlaD are considered to possess no orthologs except for the putative products of the well-conserved ylaABCD operon of B. licheniformis (Rey et al., 2004). Identical amino acid sequences between B. subtilis and B. licheniformis are 37%, 44%, 62% and 46% in YlaA, YlaB, YlaC and YlaD, respectively.

In this communication, we have examined the regulation of transcription of the ylaABCD operon of B. subtilis by the lacZ reporter assay and Northern analysis. Trancription initiation sites of the ylaABCD operon were also determined and the promoter sequences were compared with those of the other ECF sigma factors.

**MATERIALS AND METHODS**

**Bacterial strains and culture media** The bacterial strains and plasmids used in this study are described in Table 1. B. subtilis Marburg 168 trpC2 was used as a wild-type strain in all experiments. These strains were grown at 37°C in Luria-Bertani (LB) broth (Sambrook et al., 1989), or in Difco sporulation medium (Schaeffer et al., 1965).

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\(^a\)Cm, chloramphenicol (5 µg/ml); Em, erythromycin (0.3 µg/ml); Km, kanamycin (5 µg/ml), for B. subtilis strains and Ap, ampicillin (50 µg/ml) for E. coli strains.

\(^b\)www://Bacillus.genome.ad.jp/
DNA manipulation Plasmid DNA purification, PCR amplification of DNA fragments, digestion of DNA with restriction enzyme, and DNA ligation were carried out as described elsewhere (Sambrook et al., 1989). Plasmid DNA was purified by polyethylene glycol precipitation of a sample of E. coli C600 cells lysed by alkali. The primers used in this study were custom made (Espec Oligo Service, Tsukuba, Japan).

Disruption of ORFs Disruption of ORFs with the integrative plasmid pMUTIN was carried out as described elsewhere (Vagner et al., 1998). PCR primer pair for disruption of ylaD was 5'-AAGAAGCTTTTTCTAGTAAGAGC/5'-GGAGGATCCGGAAGATAAGGC. The cells were harvested immediately before growth. Diamide was added at a final concentration of 1 mM. The resulting transformant was designated as amyE::P<sub>lacZ</sub>-lacZ. Primer pair for the promoter cloning was 5'-GAA-GAATTCCGGTGCTGAAGAAGAAGGCGAAGTGTATCAACAAGGCTGC/5'-GGAGGATCCCTGCGCTAAGTCCTTGGC.

Transformation Competent B. subtilis cells were prepared in Spizizen's minimal glucose medium, and transformation was carried out as described elsewhere (Ohshima et al., 2002). Transformation of E. coli was performed as described elsewhere (Groth et al., 1996).

RNA purification Total RNA was isolated from cells as described previously (Yoshida et al., 2000) with minor modifications.

Northern blot analysis RNA was separated by electrophoresis, and detected with a digoxigenin (DIG)-labeled RNA probe as described elsewhere (Asai et al., 2003). The DNA region containing the structure genes of the ylaABCD operon and their Shine-Dalgarno sequences was amplified by PCR using chromosomal DNA from strain 168 and the primer pairs listed below. For ylaB, 5'-GTCGTCGACACAAGAGGTGAAAAGTC/5'-GGAGGATCCGGTGCTGAAGAAGAAGGCGAAGTGTATCAACAAGGCTGC; for ylaBC, 5'-GTCGTCGACACAAGAGGTGAAAAGTC/5'-GGAGGATCCGGTGCTGAAGAAGAAGGCGAAGTGTATCAACAAGGCTGC, and for ylaC, 5'-GTCGTCGACACAAGAGGTGAAAAGTC/5'-GGAGGATCCGGTGCTGAAGAAGAAGGCGAAGTGTATCAACAAGGCTGC.

β-Gal assay The activity of β-galactosidase was measured as described elsewhere (Yoshida et al., 2000) with following modifications. The cells were collected by centrifugation and stored at -20°C. The cells were suspended in 250 µl Z buffer (60 mM Na<sub>2</sub>HPO<sub>4</sub>, 40 mM NaH<sub>2</sub>PO<sub>4</sub>, 10 mM KCl, 1 mM MgSO<sub>4</sub> and 50 mM mercaptoethanol) containing 25 µg βββββββββbeta-ββββββββββ-galactosidase/ml (Sigma, St.Louis, USA) and 10 µg β-galactosidase/ml (Sigma), and incubated at 37°C for 20 min. After centrifugation (14000rpm, 5 min), the supernatants were used for spectrophotometric β-Gal assays and protein assays. 200 µl of the supernatants were transferred into new microtubes. 300 µl Z buffer and 200 µl o-nitrophenyl-β-β-galactopyranoside (ONPG) were added. The mixture was incubated at 28°C. β-Gal reaction was stopped by the addition of 500 µl 1M Na<sub>2</sub>CO<sub>3</sub>. Protein concentrations were determined using the Bio-Rad Protein Assay kit. β-galactosidase activity was expressed in nmol o-nitrophenol produced/min/mg of protein.
Determination of the start sites of transcription of ylaC operon  Total RNA was extracted from cells of strain BSU37 (amyE::P_{ylaC-lacZ}) carrying pDG148-ylaC, which accepted 1mM IPTG for 1 hr, and from cells of BSU62 (amyE::P_{ylaD-lacZ}) treated with 1mM diamide for 15 min, for determination of transcription initiation sites of ylaA and ylaC promoters respectively. The primer extension was carried out as described elsewhere (Takamatsu et al., 2000) with digoxigenin labeled primer DNA (5'-TGTATCAAAAGCTGGGGGATC) and reverse transcriptase (Invitrogen Super Script™ II RNase H^- Reverse Transcriptase). For the 5' RACE method (Eichenberger et al., 2003), an aliquot of 50 µg of total RNAs extracted as describe above were reverse transcribed using SuperScript™II RT (Invitrogen, San Diego, USA) in the presence of 70 pmol of the primers, 5'-GGAGGATCCGTCAGCAGCATAATC and 5'-GGAGGATCCATCCGCTCCTCTCTCTC- TAC for ylaA and ylaC transcription initiation site determination, respectively. 10 µl 150 mM NaOH was added, and the mixture was incubated at 65°C for 1 hr for the degradation of RNA. The sample was purified using a MinElute spin column (Qiagen, Hilden, Germany). A homopolymeric A-tail was added to the 3'-end of the cDNA with terminal transferase (Roche), and the sample was purified using a MinElute spin column again. The tailed cDNAs served as templates for PCR in the presence of a poly dT primer 5'-TTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTT
**ylaABCD operon of B. subtilis**

Promoter responding to oxidative stress.

**Transcription initiation sites of the distal and internal promoter of the ylaABCD operon**

Transcription initiation sites of distal and internal promoters were determined by the primer extension method (Fig. 3 A, B). Both initiation sites were Gs, 24 bases upstream of the initiation codon TTG of ylaA and the third base of the initiation codon ATG of ylaC. Transcription initiation sites determined by the 5' RACE method were the same as described above (data not shown), although this method gave start sites at TGs. The sequences of –35

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**Fig. 1.** Northern analysis of the transcripts of the ylaABCD operon. (A) Transcription was induced by over-expression of ylaC. The ylaC was fused to P\textsubscript{spac} promoter on the plasmid pDG148 and over-expressed with IPTG(1mM). –; without IPTG. +; with IPTG. RNA probes were #1(ylaA) and #4(ylaD). (B) Transcription was induced by diamide (1 mM) addition. RNAs were extracted from the cells grown in LB broth containing diamide for 30 min. RNA probe were #1(ylaA), #2(ylaB), #3(ylaC), and #4(ylaD). Filled arrow heads indicate transcripts. Open arrow heads indicate the positions of 23S and 16S rRNAs. Configuration of ylaABCD operon is shown. Black bars indicate the regions of the RNA probes.

**Fig. 2.** Transcription from the internal promoter was dependent on Spx. Northern analysis of the RNA extracted from wild type (A) or spx deficient (spx::pMUTIN) (B) cells at 0, 15 and 30 min after 1 mM diamide addition. RNA probe was #3(ylaC) described in the legend of Fig. 1. Black arrows indicate transcripts from the internal promoter of ylaABCD operon.
(TGAAAC) and –10(TGTCTA) region of the distal promoter are very similar to those recognized by ECF sigmas (Helmann, 2002; Helman & Price, 2002; Huang et al., 1998; Huang et al., 1999, Qui & Helmann, 2001), while –35(TTGGCC) and –10(TGGTTTTAT) of the internal promoter are similar to –35(TTGACA) and –10(TGN-TATAAT) of sigA promoter (Helmann and Moran, 2002).

Although no functional analysis of the orthologus ylaABCD operon of B.licheniformis has been described, the distal promoter sequences –35(TGAAAC), 16 base spacer, –10(TGTCTA) are conserved even in B.licheniformis. While the transcription initiation site of the ylaA promoter of B.licheniformis is not known, DNA sequences TGAGGCTTAAT containing the initiation site of the B. subtilis ylaA operon is strictly conserved in B.licheniformis. On the other hand, DNA sequences of the internal promoter –35(TTGGCC), 15 base spacer, –10(TGGTTTTAT) of B. subtilis are also conserved in B.licheniformis, where the putative internal promoter is –35(CTGTGC), 15 base spacer, –10(TGGTTTTTT). The 15 base spacer sequences are the same in both bacteria. Therefore, the transcription initiation site of the internal promoter must probably be conserved in B.licheniformis.

**The ylaC was not translated when transcription was induced from the internal promoter of the ylaABCD operon** In the internal promoter, transcription starts from the third base of the initiation codon ATG of ylaC. To determine whether or not ylaC-translation initiates from the second initiation codon, the presence of the YlaC protein was analyzed, by Western blotting and with FLAG Tag added to its C-terminal end. When the transcription of the whole operon was induced by over expression of ylaC, YlaC-FLAG was detected (Fig. 4B), but it was not detected during oxidative stress (diamide)-induced transcription from the internal promoter (Fig. 4A). Therefore it seemed very probable that the +1 internal transcription start site is within ylaC, and YlaC is not translated.

**YlaD is an anti-sigma factor of YlaC** YlaD possesses an oxidative stress sensing domain with one histidine and three cysteine residues complexed with zinc metal. The domain is well conserved in the anti-ECF sigma factors, RsrA of SigR of S. coelicolor and YbbM of SigW of B. subtilis (Paget et al., 2001). To see whether or not YlaD is the anti-YlaC, we performed experiments with over-expression or disruption of ylaD.

Over-expression of both ylaC and ylaD resulted in reduced induction of the whole ylaABCD operon compared with the induction by over-expression of ylaC alone (Fig. 5A). To our surprise, over-expression of both ylaB and ylaC strongly enhanced the whole ylaABCD operon expression because of probable translational coupling (Inokuchi et al., 2000) as the translational termination codon of ylaB is adjacent to the translational initiation.

![Fig. 3. Transcription initiation sites of the distal and the internal promoters of the ylaABCD operon. Transcription initiation sites were determined by the primer extension method, and initiation sites are indicated by asterisk. (A) Reverse transcription was performed with RNA extracted from cells in which ylaC was over-expressed. (B) Reverse transcription was performed with RNA extracted from cells treated with diamide (1 mM) for 30 min.](image_url)
The codon of ylaC (TAATG). Even in this ylaABCD operon induction by over-expression of ylaBC, over-expression of ylaD in addition to ylaBC reduced expression of the whole ylaABCD operon (Fig. 5A). This reduction was small but significant. Therefore we considered that YlaD negatively regulated YlaC function, provided that cloned ylaBCD and ylaBC were over-expressed equally well. Over-expression of ylaD did not result in depression of
diamide-induced transcription of ylaCD operon (data not shown).

Disruption of ylaD, on the other hand, resulted in enhanced expression of the whole ylaABCD operon (Fig. 5B). Expression of ylaABCD operon was very low, but enhancement by ylaD-disruption was significant.

Although direct interaction only between YlaB and YlaD was detected by the yeast two-hybrid system and no interaction was found between YlaC and YlaD (Yoshimura et al., 2004), YlaD seemed to function as an anti-YlaC factor, because disruption of ylaD resulted in an enhanced transcription of the whole operon, and over-expression of ylaD reduced the YlaC dependent ylaABCD operon expression.

As over-expression of ylaB in addition to ylaC lightened reduction of the whole ylaABCD operon expression by YlaD, YlaB might function as modulator of YlaD. Direct interaction of YlaD and YlaB may support this.

Although YlaD has an oxidative stress sensing domain, oxidative stress did not activate YlaC that led to induction of the whole ylaABCD operon. It may be possible that the ylaABCD operon of B. subtilis is losing its function or that the transcript from the internal promoter or its translational product possesses some unknown function.

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REFERENCES


