Bacillus subtilis comZ (yjzA) Negatively Affects Expression of comG but Not comK

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The yjzA open reading frame, along with med, constitutes an operon. Disruption of yjzA caused a five-fold enhancement of comG expression, thereby leading to a three-fold-higher transformation efficiency. The expression of comK and the other three late competence operons was not affected significantly in the yjzA-deficient mutant.

For Bacillus subtilis, competence is the ability to bind, process, and take up exogenous DNA (5, 6). Nutrient depletion and high cell density cause development of competence, which is a highly regulated differentiation process (7). The competence transcription factor, ComK, triggers transcription of the late competence operons, comC, comE, comF, and comG (5, 6, 10). The comG operon encodes seven proteins essential for competence (1). The ComGA protein with nucleotide-binding sites is located at the inner face of the cell membrane, while ComGB resembles PilB and PilC, respectively, from Pseudomonas aeruginosa, which are required for the assembly of type 4 pili (5, 6). ComGC, ComGD, ComGE, and ComGG are processed by the peptidase ComC and are localized at the outer surface of the cell membrane (2, 4). They have N-terminal sequences resembling the cleavage sites of type 4 prepilin proteins (1). It has been postulated that ComEA is responsible for initial DNA binding and that the complex composed of ComGC, ComGD, ComGE, and ComGG would permit access of the cell wall structure (4, 6, 18). A polytopic membrane protein, ComEC, which may be all or part of an aequous channel for DNA translocation, is required for DNA transport across the cell membrane. An integral membrane protein with a nucleotide-binding site, ComFA, resembles DNA helicase and may translocate DNA by ATP hydrolysis (5, 6, 11, 13, 14).

yjzA encodes a 63-amino-acid protein and along with the upstream med gene constitutes an operon. The med-yjzA operon is preceded by two putative ComK boxes (AAAC-N5-ATTANc-AAAA-Nc-TTTT [Fig. 1] [10, 12, 17]). Disruption of meca that results in overproduction of ComK causes hypertranscription of this operon (17). In turn, Med is involved in positive regulation of ComK (17), indicating that med and comK constitute a regulatory circuit. Thus, it may be reasonable to assume that yjzA might also be involved in competence regulation. To examine the effects of disruption of yjzA on the expression of comK and the late com operons, we constructed strains carrying both a yjzA disruption and a fusion of lacZ with each of the genes in the operons and determined their expression patterns.

Strains carrying transcriptional fusions between comC or comE and lacZ were made as follows. The PCR products obtained by using the primer pair comC-U (5’-CTTAGATCTA GGAGTTAATGTTGCGCGCA-3’) and comC-D (5’-ATC AAGCTTTAAAAGCATCCGCTCCGGC-3’) and the primer pair comE-U (5’-ATCAGATCTACGGATACCGAGCCATCTAGG-3’) and comE-D (5’-CTTAAGCTTCCGAATTGTATACC CGGC-3’) were digested with BglII and HincIII and cloned between the BamHI and HindIII sites of pMutinIII (19). The resultant lacZ fusion plasmids were transformed into B. subtilis CU741. Next, total DNA from strain OMD43 carrying yjzA:Tc’ (17) was transformed into the strains carrying the fusions. Cells were grown in modified competence (MC) medium, and β-galactosidase activity was determined as previously described (15, 17).

Whether yjzA was disrupted or not, expression of a transcriptional comK-lacZ fusion did not change (Fig. 2A). This suggests that the expression of the ComK-regulated late com operons may not be affected in the yjzA mutant strain. Surprisingly, however, we observed that disruption of yjzA caused a five-fold enhancement of comG-lacZ expression, although no significant change was observed for the comC- and comE-lacZ fusions in the yjzA mutant strain (Fig. 2B, C, and D). The expression of comF-lacZ was reduced to 70% of that observed in wild-type cells (Fig. 2E). This phenomenon was observed reproducibly, although it is not yet known whether the decrease in comF expression plays any role in competence.

We observed that the expression of comG-lacZ was still strictly dependent on ComK in the yjzA mutant strain (data not shown). Since the comK gene is expressed in only competent cells, which are a subpopulation of the culture, enhancement of comG-lacZ expression would occur in the competent subpopulation. Thus, the molecular ratio of the ComG proteins to the other late Com proteins should be raised in a single competent cell. The degU gene is required for efficient comK transcription and thus for the expression of late com operons (8, 9, 16, 20). The expression of comG-lacZ was almost abolished when degU was disrupted (Fig. 2B). Introduction of disruption of both yjzA and degU to the strain carrying comG-lacZ resulted in partial recovery of comG-lacZ expression, indicating that the effect of the yjzA mutation on expression of comG-lacZ is dependent on a very small amount of ComK. YjzA may somehow repress transcription of the comG operon directly or indirectly, although the precise mode of action of YjzA is not yet known. It is worthy to note that an analysis of the amino acid sequence of YjzA revealed the presence of a leucine-zipper motif (L10-7...
amino acids [aa]-L17-7 aa-L24-7 aa-L31); hence, YjzA may constitute a dimer.

These results raised the question of whether transformation efficiency in the yjzA mutant is higher than that of the wild-type strain. To examine the possible relationship between the transformation efficiency and cellular concentration of the YjzA protein, we used the OMM100 strain, in which yjzA transcription is blocked by a vector insertion and an isopropyl-β-D-thiogalactopyranoside (IPTG)-inducible Pspac promoter is located upstream from the intact yjzA open reading frame (Fig. 1) (17). Transformation efficiency was determined by measuring the number of transformants (Leu<sup>+</sup>) obtained compared with the total cell number. Transformation efficiency in the yjzA strain was increased at least three-fold compared to that in the wild-type strain in three independent experiments (Table 1). Moreover, when increasing concentrations of IPTG were used for the OMM100 culture, the efficiency of transformation was gradually reduced to that of the wild-type strain. This may be due to the stimulation of transcription of yjzA by addition of IPTG. It is interesting that at the highest concentration of

FIG. 1. Structure of the med-yjzA operon. The arrows and stem-loop structures indicate promoters and terminators, respectively. Boxes, except for the ComK box, show open reading frames. The med-yjzA operon in the chromosome of wild-type CU741 and the chromosome structure around the med-yjzA operon in OMM100 (17) are shown.

FIG. 2. Disruption of yjzA resulted in enhanced expression of comG-lacZ. Cells were grown in MC medium containing appropriate antibiotics, and β-galactosidase activities were determined as described previously (15). The activities are shown in Miller units. The numbers on the x-axis represent the growth time in hours relative to the end of the vegetative growth (T<sub>0</sub>). The transcriptional comG-lacZ fusion in OGM100 was derived from BD1512 (1). comC-lacZ and comE-lacZ were constructed in this study (see text). The translational comF-lacZ fusion was a gift from D. Dubnau (13). All the strains are derivatives from CU741. (A) comK-lacZ fusion expression. ○, OCM100 (comK-lacZ) (17); ◊, OCM116 (comK-lacZ yjzA). (B) comG-lacZ fusion expression. ○, OGM100 (comG-lacZ) (16); ◊, OGM108 (comG-lacZ yjzA). (C) comC-lacZ fusion expression. ○, OGM111 (comC-lacZ); ◊, OGM112 (comC-lacZ yjzA). (D) comE-lacZ fusion expression. ○, OGM113 (comE-lacZ); ◊, OGM114 (comE-lacZ yjzA). (E) comF-lacZ fusion expression. ○, OGM115 (comF-lacZ); ◊, OGM116 (comF-lacZ yjzA).
TABLE 1. Disruption of yjzA-stimulated transformation efficiency

<table>
<thead>
<tr>
<th>Expt no.</th>
<th>CU741 yjzA mutant</th>
<th>OMM100 grown with an IPTG concentration of:</th>
</tr>
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<tbody>
<tr>
<td></td>
<td></td>
<td>0 nM</td>
</tr>
<tr>
<td>1</td>
<td>0.33</td>
<td>1.6</td>
</tr>
<tr>
<td>2</td>
<td>0.81</td>
<td>1.9</td>
</tr>
<tr>
<td>3</td>
<td>0.21</td>
<td>1.5</td>
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* DNA was added to the cell cultures at T_c. After the transformation the cells were subjected to serial dilutions, i.e., 10^{-2}, 10^{-3}, and 10^{-4}. Each diluted fraction was plated onto two minimum medium plates containing tryptophan alone, and then colonies were counted. Total cell numbers were counted by plating the culture onto three minimum medium plates containing tryptophan and leucine after 10^{-1} dilution. Results obtained by three independent experiments are shown. Values are percentages.

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REFERENCES