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Expression of the *Bacillus subtilis spoIVCA* Gene, Which Encodes a Site-Specific Recombinase, Depends on the *spoIIGB* Product

TSUTOMU SATO, KENJI HARADA, YASUHIKO OHTA, AND YASUO KOBAYASHI*

Department of Applied Biological Science, Faculty of Agriculture, Tokyo University of Agriculture and Technology, Fuchu 183, Japan

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The *Bacillus subtilis spoIVCA* gene encodes a site-specific recombinase which creates a *sigK* gene by DNA rearrangement. We have determined the transcription initiation point of the *spoIVCA* gene and found that (i) the *spoIVCA* promoter contains sequences which are similar to −10 and −35 regions of promoters recognized by σ^E and (ii) mutation of *spoIIGB*, which encodes pro-σ^E, blocked the expression of *spoIVCA*.

Sporulation of *Bacillus subtilis* initiates in response to nutrient deprivation. One of the key points in the morphological differentiation is asymmetrical septum formation, which partitions the sporangium into two compartments, the mother cell and the forespore. During sporulation, various sets of genes are turned on sequentially by mechanisms that require synthesis and activation of several RNA polymerase sigma factors. In the mother cell, DNA rearrangement occurs to create the *sigK* gene, which codes for a mother cell-specific sigma factor, σ^K (4, 12, 16). The *sigK* gene is a composite of two truncated genes called *spoIVCB* (5, 16), which encodes the N-terminal portion of σ^K, and *spoIIIC* (2), which encodes the C-terminal portion of the factor. *spoIVCB* and *spoIIIC* are joined together in frame by excision of a 42-kb intervening DNA element (4, 16) as a covalently closed circular DNA (11a). The *spoIVCA* gene, located adjacent to *spoIVCB*, encodes a polypeptide of 500 amino acid residues with a calculated molecular weight of 57,481 (12). The amino acid sequence of the N-terminal portion of this protein is homologous to the Hin invertase and TnpR resolvase families of site-specific recombinases (12). Popham and Stragier (9) showed that the SpoIVCA protein binds specifically to the 21-bp repeats flanking the recombination sites. *spoIVCA* expression reaches a maximum at T_3 (3 h after the initiation of sporulation), coinciding with the occurrence of the DNA rearrangement (12). We now show that *spoIVCA* expression depends on the *spoIIGB* product, σ^E.

Mapping the 5′ terminus of *spoIVCA* mRNA. The 5′ terminus of *spoIVCA* mRNA was mapped by extension of an 18-nucleotide-long synthetic primer (3′ CCCATAGCTGGC TCCTTG 5′), which was designed to anneal to the 5′-terminal part of *spoIVCA* mRNA (see Fig. 1B). The results of electrophoresis showed that the 5′ terminus (+1) of *spoIVCA* T_3 mRNA was 111 bp upstream from the translation initiation codon (Fig. 1). A low-resolution S1 nuclease mapping experiment gave a similar result for the 5′ terminus (12). The nucleotide sequences of the −35 (cTCATtTgg) and −10 (CATAgcAT) regions of the *spoIVCA* promoter are similar to the consensus sequences for the −35 G(G/T)(C/a)AT(A/g)(T/a)(T/a)(t/c) and −10 (C/a)ATACA(a/c)T recognition sequences for σ^E-controlled promoters (Fig. 2).

Expression of a *spoIVCA-lacZ* transcriptional fusion gene in *spo* mutants. To determine the dependence pattern of *spoIVCA* expression, we constructed a specialized transducing phage, ϕCMIVCA, carrying a transcriptional *spoIVCA-lacZ* fusion. *lacZ* fusion vector pSZ1, containing the Shine-Dalgarno sequence and the first 17 codons of the *aprE* gene preceding the truncated *lacZ* gene (18), was used to construct a transcriptional *spoIVCA-lacZ* fusion gene. We first isolated a 628-bp (from −441 to +187) *Hpa*II-*Hae*III fragment containing the *spoIVCA* promoter from a 3.6-kb *Eco*RI fragment (12). In order to insert the 628-bp *Hpa*II-*Hae*III fragment into the *Pst*I-*Eco*RI site located upstream of the Shine-Dalgarno sequence of pSZ1, plasmid pUCAP was constructed by inserting the 628-bp *Hpa*II-*Hae*III fragment into the *Acc*I-*Sma*I site of

TABLE 1. *B. subtilis* strains used

Strain	Genotype	Reference or source[a]
JH642	*trpC2 pheA1*	J. A. Hoch
UOT0531	*trpC2 metB51 leuA8 nonB1*	F. Kawamura
1.5	*spoIIAC1 trpC2*	J. Errington
1S49	*spoIIB131 trpC2*	BGSC[b]
1S33	*spoIID66 rpoB2 trpC2*	BGSC
1S35	*spoIIE64 rpoB2 trpC2*	BGSC
1S42	*spoIIIA35 metC2 tal-1*	BGSC
1S39	*spoIIID83 trpC2*	BGSC
1S63	*spoIIIE36 trpC2*	BGSC
1S27	*spoIIIJ87* (formerly *spo0J87*) *metC2 tal-1*	BGSC
1S47	*spoIVCA133 trpC2*	BGSC
KH2	*spoIIAC1 trpC2*	1.5 $\overset{co}{\rightarrow}$ JH642
KH3	*spoIIB131 trpC2*	1S49 $\overset{co}{\rightarrow}$ JH642
KH4	*spoIID66 trpC2*	1S33 $\overset{co}{\rightarrow}$ JH642
KH5	*spoIIE64 trpC2*	IS35 $\overset{co}{\rightarrow}$JH642
OD8701	*spoIIGAΔ trpC2 pheA1*	6
KH6	*spoIIGB::cat*[c] *trpC2 pheA1*	pCXIIGB $\overset{dtf}{\rightarrow}$ JH642
KH7	*spoIIIA35 metC3 pheA1*	1S42 $\overset{co}{\rightarrow}$ JH642
KH8	*spoIIID83 trpC2*	1S39 $\overset{co}{\rightarrow}$ JH642
KH9	*spoIIIE36 trpC2*	1S63 $\overset{co}{\rightarrow}$ JH642
OD8603	*spoIIIGΔ trpC2 pheA1*	7
KH1	*spoIIIJ87 pheA1*	1S27 $\overset{co}{\rightarrow}$ JH642
KH10	*spoIVCA133 trpC2*	1S47 $\overset{co}{\rightarrow}$ JH642

[a] co, congression or cotransformation of unlinked markers; tf, transformation; donor→recipient.
[b] BGSC, *Bacillus* Genetic Stock Center, Ohio State University.
[c] The symbol *spoIIGB::cat* refers to the chloramphenicol acetyltransferase gene (*cat*) of pCXIIGB integrated at the *spoIIGB* locus.
[d] pCXIIGB is a derivative of an integrational plasmid, pCX19 (18), carrying a 198-bp *Hin*dIII *spoIIGB* fragment.

* Corresponding author. Phone: 81-423-34-5988. Fax: 81-423-34-5843.

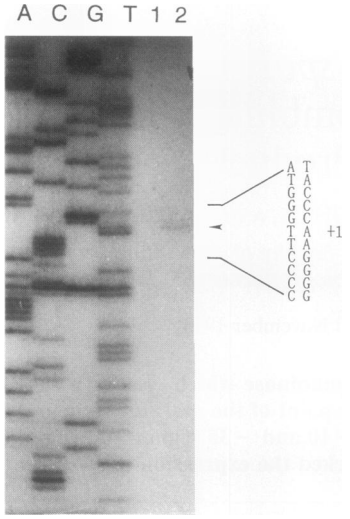

FIG. 1. Primer extension mapping of the *spoIVCA* transcript. The primer extension reaction has been described by Sambrook et al. (10). (A) Autoradiograph of denatured 6% polyacrylamide–urea gel. RNA was isolated from the vegetative (lane 1) and T_3 (lane 2) cells of JH642 growing in Schaeffer sporulation medium (13). Dideoxynucleotide sequencing reactions (11) were run with the same primer, which was used to map the 5′ end of the *spoIVCA* mRNA (Fig. 1B). The complementary base of the start site is indicated by an arrowhead (+1). (B) Nucleotide sequence of the upstream region of *spoIVCA*. The transcription start site is indicated by +1, and the putative −10 and −35 regions of the *spoIVCA* promoter are underlined with thick lines. The Shine-Dalgarno sequence is underlined with a thin line, and the putative initiation codon is boxed. The nucleotide sequence of the primer used for the primer extension experiment is shown.

pUC118. pUCAP was digested with *Pst*I and *Eco*RI, and the isolated 655-bp *Pst*I-*Eco*RI fragment was inserted into pSZ1. This plasmid was designated pSZIVCA. To place a *Bam*HI site downstream of the *spoIVCA-lacZ* fusion gene, plasmid pUZ1 (18), which contained a *lacZ* gene whose 3′ flanking region has a *Bam*HI site, was digested with *Pst*I and *Ban*III to remove the N-terminal region of the *lacZ* gene and then this region was replaced by the corresponding *Pst*I-*Ban*III fragment derived from pSZIVCA. This fragment carries the N-terminal portion of the *spoIVCA-lacZ* fusion gene. This plasmid was designated pUZIVCA1. A 3.5-kb *Bgl*II-*Bam*HI fragment, containing a *spoIVCA-lacZ* gene isolated from pUZIVCA1, and *Bam*HI-digested ϕCM DNA (14) were mixed, ligated, and inserted by prophage transformation into the ϕ105 prophage lysogenized in *B. subtilis* UOT0531 cells to construct a *spoIVCA-lacZ*-transducing phage, ϕCMIVCA. Then ϕCMIVCA was lysogenized in various *spo* mutants (Table 1), and the β-galactosidase activity of each strain was measured during sporulation (8) (Fig. 3). Expression of *spoIVCA-lacZ* was blocked in *spo0A*, *-0B*, *-0E*, *-0F*, and *-0H* mutants (data not shown). Mutations in the stage II loci *spoIIAC*, *-IIE*, *-IIGA*, and *-IIGB* also blocked

	"-35" region										"-10" region								
	-36	-35	-34	-33	-32	-31	-30	-29	-28		-14	-13	-12	-11	-10	-9	-8	-7
A	2	1	6	16	3	13	6	6	2	A	6	19	0	20	3	12	9	3
T	4	9	2	2	15	2	10	11	8	T	0	0	20	0	4	0	4	16
G	10	10	0	2	0	5	2	1	3	G	0	0	0	0	3	5	0	0
C	4	0	12	0	2	0	2	2	7	C	14	1	0	0	10	3	7	1
	G	G/T	C/a	A		T	A/g	T/a	T/a	t/c		C/a	A	T	A	C	A	a/c	T

spoIVCA promoter

c T C A T t T g g C A T A g c A T

FIG. 2. Comparison of sequences of promoters directed by σ^E. The middle line shows the consensus σ^E promoter sequence. A base was included in the consensus if it was found in at least 5 of 20 promoters. Small letters in the consensus indicate less-well-conserved bases (occurs in 5 to 9 of 20 promoters, except for the bases at −35 and −9). The numbers above the consensus indicate the number of occurrences in 20 promoters. The figure is an updated version of that shown in reference 3, incorporating the *orf60* (1), *spoVID* (1), *spoIIM* (15), *spoVE* P2 (17), and *spoIVCA* promoters.

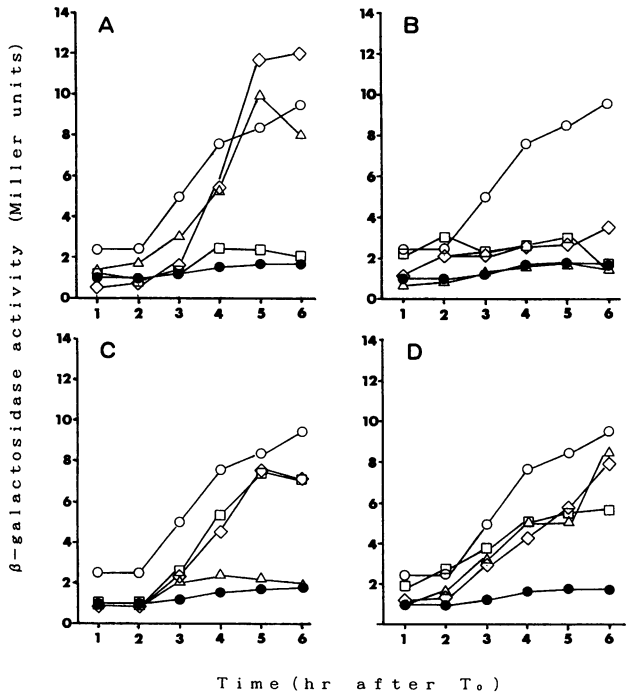

FIG. 3. *spoIVCA*-directed β-galactosidase synthesis during sporulation in sporulation mutants. β-Galactosidase activities of JH642 lysogenized with ϕCMIVCA and ϕCM (14) are shown by open and closed circles, respectively. (A) KH2 (*spoIIAC1*, □), KH3 (*spoIIB131*, △), and KH4 (*spoIID66*, ◇). (B) KH5 (*spoIIE64*, □), OD8701 (*spoIIGAΔ*, △), and KH6 (*spoIIGB::cat*, ◇). (C) KH7 (*spoIIIA35*, □), KH8 (*spoIIID83*, △), and KH9 (*spoIIIE36*, ◇). (D) OD8603 (*spoIIIGΔ*, □), KH1 (*spoIIIJ87*, △), and KH10 (*spoIVCA133*, ◇).

spoIVCA-lacZ expression. Expression was not blocked in *spoIIB* and *spoIID* mutants. Mutations in the stage III and stage IV loci *spoIIIA*, *-IIIE*, *-IIIG*, *-IIIJ*, and *-IVCA* did not block *spoIVCA-lacZ* expression, but a *spoIIID* mutation did block expression. These results indicate that *spoIVCA*-directed β-galactosidase synthesis is prevented by all mutations that block the expression of *spoIIGB*, which encodes σ^E, and suggest that *spoIVCA* expression depends on σ^E. Preliminary in vitro experiments with purified $E\sigma^E$ holoenzyme and *spoIVCA* template DNA showed that *spoIVCA* was transcribed by $E\sigma^E$. In addition, our β-galactosidase assay results suggest that the *spoIIID* product is required for *spoIVCA* expression. Kunkel et al. (4) suggested two possibilities for the role of SpoIIID in the recombination process. One is that SpoIIID is required for *spoIVCA* transcription, and the other is that SpoIIID directly participates in the recombination event. Our results suggest that SpoIIID is required for *spoIVCA* transcription rather than or in addition to a direct role for SpoIIID in the recombination process.

Nucleotide sequence accession number. The nucleotide sequence of the upstream region of *spoIVCA* has been assigned DDBJ accession number D17339.

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