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Early sporulation gene *spo0F*: Nucleotide sequence and analysis of gene product

(recombinant DNA/*in vitro* transcription/maxicell)

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ABSTRACT **We have determined the sequence of a 1,162-base-pair DNA fragment containing a *spo0F* gene which is required for an early stage of sporulation in *Bacillus subtilis*. The sequence has only one long open reading frame consisting of 173 codons, which has been confirmed to be the *spo0F* cistron by DNA-mediated transformation and *in vitro* transcription. In UV-irradiated "maxicells" containing pUBSF13, the plasmid that carries cloned *spo0F* gene, we have observed the synthesis of a 20-kilodalton polypeptide that is absent from cells carrying a vector plasmid pUB110. The molecular weight of this protein is in agreement with the calculated molecular weight of the *spo0F* gene product (M_r, 19,065). The putative promoter sequences of *spo0F* gene were 5′ T-A-T-A-A-T 3′ at −10 and 5′ T-T-G-A-T-T 3′ at −35. An octamer sequence, 5′ A-A-A-G-G-A-G-G 3′, situated 8 base pairs prior to the initiation codon was found to be perfectly complementary with the 3′ end of 16S ribosomal RNA. This result offers additional evidence for the proposal by Rabinowitz's group that an extensive mRNA–rRNA interaction is a requirement for efficient translation by *B. subtilis* ribosomes.**

One of the primitive forms of differentiation is represented by the formation of spores in certain bacteria. The formation of a spore follows a sequence of morphological and biochemical events. The process of sporulation in *Bacillus subtilis* has been investigated in detail (1, 2). A large number of sporulation-defective mutants have been isolated and their mutations have been mapped (3). These mutants have been classified into five types (*spo0*, *spoII*, *spoIII*, *spoIV*, and *spoV*) according to the stage at which sporulation of the mutant is blocked. Among these mutants, the *spo0* type is particularly interesting because initiation of the sporulation is dependent upon the products of the *spo0* genes. At least nine loci of *spo0* genes have been mapped (4) and a mutation in any one of these genes blocks sporulation before the morphological change characteristic of early spore development. Despite their crucial role in development, however, little is known about the *spo0* genes and how they control the initial events of spore formation.

We recently developed a method, called "prophage transformation," for constructing specialized transducing phages of ρ11 (5) and φ105(6). To study the initiation of sporulation, we have used these phages to clone the early sporulation gene *spo0F*. The activity to transform *spo0F* was found in a 2.2-kilobase-pair (kb) *Eco*RI fragment of the transducing phage DNAs (7, 8). These transducing phages were designated ρ11*spo0F* and φ105*spo0F*; upon lysogenization they could restore the ability of mutant JH649 (*spo0F221*) to sporulate. In contrast to the phage vectors, which exist as a single prophage genome, plasmid vectors such as pUB110 are known to reside as multiple copies (9). The multicopy plasmid pUBSF13 harboring the entire *spo0F* gene did not restore the ability of mutant JH649 to sporulate. This result indicated that the sporulation was inhibited, for an unknown reason, by the multi-copy plasmid (8).

Rhaese *et al.* (10) proposed that the *spo0F* gene encodes a synthetase for a highly phosphorylated nucleotide adenosine 5′,3′(2′)bistriphosphate. However, the synthetase protein has not been isolated, and the true function of the *spo0F* gene in the early stage of the sporulation has not been discovered. In order to clarify the initiation process of sporulation, it is necessary to study the cloned early sporulation gene at the molecular level. In the present communication we report the location of the *spo0F* gene in a 2.2-kb *Eco*RI fragment, its direction of transcription, and its sequence.

MATERIALS AND METHODS

Bacterial Strains, Bacteriophage, and Plasmids. *B. subtilis* JH649 (*trpC2*, *pheA1*, *spo0F221*), which was supplied by J. Hoch (Scripps Clinic and Research Foundation, La Jolla, CA), was used as a host strain for a temperate phage φ105*spo0F*. The recombinant phage φ105*spo0F* which carries the *spo0F* gene was constructed by prophage transformation method as described (7, 8, 11). *B. subtilis* UOT0277 (*hisA1*, *metB5*, *recE4*, *nonB1*) was used as a host for plasmids. The recombinant plasmid pUBSF13 was constructed by inserting the *spo0F*-containing *Eco*RI fragment (0F fragment) of φ105*spo0F* into pUB110 (8). Deletion derivatives pUBSFΔB, pUBSFΔH, and pUBSFΔS were constructed *in vitro* by religation after the cleavage of pUBSF13 with *Bcl* I, *Hin*dIII, and *Sac* I, respectively.

Restriction Enzyme Analysis. The recombinant phage φ105*spo0F* was used as a source of DNA for restriction analysis and DNA sequence determination. After *Eco*RI cleavage, fragments were separated by electrophoresis on 0.8% agarose gel and a 2.2-kb *Eco*RI fragment which carried the *spo0F* gene was eluted according to the procedure of Yang *et al.* (12). The eluted DNA was further purified by DEAE-cellulose chromatography according to the procedure of Smith (13). The restriction map was constructed by digesting the 0F fragment with various restriction endonucleases. Restriction enzymes were purchased either from Bethesda Research Laboratories or Takara Shuzo (Kyoto, Japan) and were used according to the manufacturers' instructions with minor modifications.

Purification of RNA Polymerase. Preparation of RNA polymerase from cells of *B. subtilis* 168 (*trpC2*), harvested in early exponential growth phase, was carried out as described (14) except that: (*i*) the precipitate from the first ammonium sulfate fraction was dialyzed against buffer A [10 mM Tris·HCl, pH 8.0/10 mM $MgCl_2$/0.1 mM EDTA/0.1 mM dithiothreitol/10% (vol/vol) glycerol] and then applied to a DEAE-Sephadex A-50 column; (*ii*) DEAE-Sephadex-purified fractions of RNA poly-

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Abbreviations: bp, base pair(s); kb, kilobase pair(s).

merase were made 70% with ammonium sulfate, and the precipitate was dissolved in and dialyzed against buffer C [10 mM Tris·HCl, pH 8.0/0.1 mM dithiothreitol/10% (vol/vol) glycerol] and the solution was applied to a DNA-cellulose column. Samples (25 μl) of the DNA-cellulose fractions were subjected to electrophoresis through a $NaDodSO_4$/polyacrylamide slab gel and the fractions containing core subunits and a σ55 subunit were used for *in vitro* transcription studies.

RNA Synthesis. The RNA polymerase was incubated at 37°C for 7 min with 0.5 μg of DNA template in a 40-μl reaction mixture containing 50 mM Tris·HCl (pH 7.8), 0.1 mM EDTA, 0.1 mM dithiothreitol, 10 mM $MgCl_2$, 0.2 mM spermidine, bovine serum albumin at 100 μg/ml, ATP, GTP, CTP, and UTP at 0.1 mM each, and 0.6 μM [α-^{32}P]ATP (10 μCi; 1 Ci = 3.7×10^{10} Bq). The reaction was stopped by mixing with an equal amount of phenol and the aqueous layer was passed through a Sephadex G-50 column (1 × 10 cm). Radioactive RNA was subjected to electrophoresis through polyacrylamide slab gels containing 7 M urea.

DNA Sequence Analysis. DNA fragments were labeled at the 5′ ends with T4 polynucleotide kinase (Bethesda Research Laboratories) and [γ-^{32}P]ATP (Amersham). DNA sequence was determined according to the procedures of Maxam and Gilbert (15). The products of the "G","G+A","T+C","C", and "A+C" reactions were electrophoresed on 0.4-mm-thick 8% or 20% polyacrylamide gels.

Preparation and Labeling of Maxicells. *B. subtilis* strain UOT0277 (*hisA1, metB5, recE4, nonB1*) was used as a host throughout the experiments. Vegetative cells containing plasmids were grown in Sterlini and Mandelstam's resuspension medium (16) supplemented with 0.2% glucose and kanamycin at 5 μg/ml. Cells were harvested by centrifugation 1 hr after the end of exponential growth and suspended in 5 ml of the fresh resuspension medium. The cell suspension was transferred to a 9-cm-diameter glass Petri dish and irradiated for 1 min at a distance of 25 cm from a 15-W germicidal lamp. After 3 hr of incubation, the cells were spun down and suspended in fresh resuspension medium. The cell suspension was incubated for 5 min at 37°C. Then 2.5 μCi of [^{14}C]leucine (340 mCi/mmol) was added and incubation was continued for 20 min. The incorporation of radioactive amino acid was terminated by adding nonradioactive leucine. The procedure of cell lysis was performed according to Hirochika *et al.* (17).

RESULTS

Location of *spo0F* Gene Within the Cloned Fragment. Previous studies have shown that the recombinant phage φ105*spo0F* contains a *B. subtilis* chromosomal DNA insert of 2.2 kb which carries the early sporulation gene *spo0F*(8). This insert was cleaved from the phage DNA with *Eco*RI and purified with an agarose gel after electrophoresis. The restriction sites of *Hin*dIII, *Bcl* I, and *Sac* I of the 0F fragment were determined as shown in Fig. 1. Recombinant plasmid pUBSF13 was constructed by inserting the 0F fragment at the single *Eco*RI site of the vector plasimd pUB110. To determine the location of the *spo0F* gene within the 0F fragment, deletion derivatives of pUBSF13 were constructed by site-specific cleavage and ligation (Fig. 1). When the 0.3-kb *Bcl* I fragment was removed from pUBSF13, the remaining DNA in the deletion plasmid pUBSFΔB lost the activity to transform *spo0F221* to Spo$^+$. Another plasmid, pUBSFΔS which lacked the 0.7-kb *Sac* I fragment, also lost the transforming activity. In contrast, deletion of the 0.3-kb *Hin*dIII fragment in pUBSFΔH did not affect the *spo0F221* transforming activity. These results indicated that at least a part of the *spo0F* gene was located between the two *Bcl* I sites of the 0F fragment (Fig. 1).

Location of the Promoters on the 0F Fragment. The initiation sites of *in vitro* transcription on the 0F fragment were mapped by analysis of RNA transcripts with various DNA templates (Fig. 2). Transcription of the 2.2-kb 0F fragment yielded two RNA species approximately 1,000 and 850 bases. When the DNA fragment lacking the internal *Bcl* I fragment (Δ*Bcl* I fragment) was used as template, only a single transcript of about 1,000 bases was detected. The largest *Hin*dIII fragment (*Hin*dIII fragment A) gave a transcript of 380 bases instead of the 1,000-base transcript. This 380-base RNA was thought to be a "run-off" product starting about 380 bp from left end of *Hin*dIII fragment A. These results suggested that the 2.2-kb 0F fragment harbored two promoters where two transcripts were initiated in opposite directions. The 850-base RNA was thought to be the transcript of *spo0F* gene, because a part of the *spo0F* gene was involved in the internal *Bcl* I fragment.

Nucleotide Sequence Analysis. Our sequence analysis strategy is outlined in Fig. 3. To investigate the coding potential of the DNA analyzed, a computer program was used to analyze the relative positions of initiation and termination codons in all six reading frames. The nucleotide sequence contains only one open reading frame long enough to code for a protein. The reading frame consists of 173 codons as shown in Fig. 4. When the 0.3-kb *Bcl* I fragment was removed from the 0F fragment, the activity to transform *spo0F221* to Spo$^+$ was lost. The internal region lying between two *Bcl* I sites, which harbored the mutation site of *spo0F221*, was involved in the open reading frame (Fig. 3).

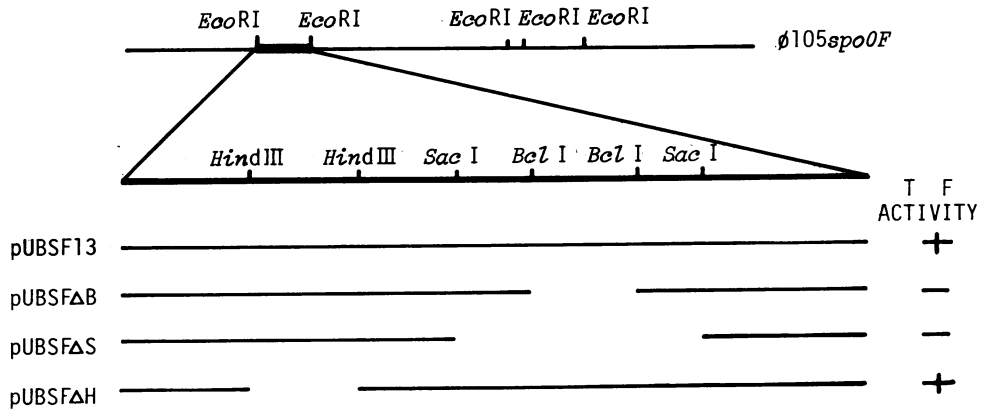

FIG. 1. Restriction endonuclease maps of cloned 0F fragment and its deletion derivatives. (*Upper*) The thick line on the φ105*spo0F* indicates the cloned *Eco*RI fragment; the restriction map is shown below it. (*Lower*) Transforming activity (TF) of pUBSF13 and *in vitro* deletion plasmids. The lines show *spo0F Eco*RI inserts of the plasmids. DNA-mediated transformation was carried out as described (18). The activity of these DNAs in transforming *spo0F221* to Spo$^+$ is shown by +.

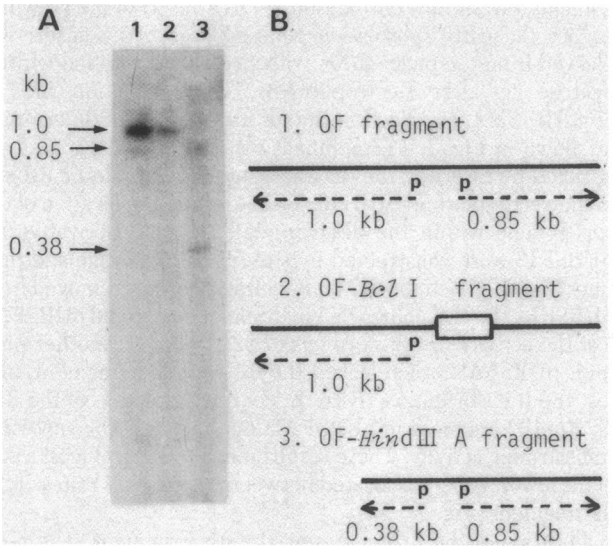

FIG. 2. *In vitro* RNA synthesis with cleaved DNA templates. RNA was labeled with [α-^{32}P]ATP and various templates. (*A*) Lanes: 1, 0F fragment (2.2 kb); 2, *Eco*RI fragment from pUBSFΔB which lost the internal *Bcl* I fragment; 3, *Hin*dIII fragment A (1.5 kb) from the 0F fragment. *E. coli* rRNAs and tRNAs were used as size markers. (*B*) DNA templates used for *in vitro* transcription experiments. Run-off transcripts are indicated by dashed lines. p, Putative promoters.

Regulatory Region of the Transcription and Translation. The principal form of the complete RNA polymerase in *B. subtilis* contains a σ subunit of 55,000 daltons known as σ 55. It has been shown by Losick and Pero (19) that promoters whose recognition is mediated by σ 55 exhibit a characteristic hexanucleotide sequence centered at position 35 and lying 10 bp upstream from the start point of transcription. These hexamers are similar to the corresponding −35 (5′ T-T-G-A-C-A 3′) and −10 (5′ T-A-T-A-A-T 3′) consensus sequences of *Escherichia coli* promoters (20). In the 5′ flanking region of the *spo0F* gene, such sequences occur in the position predicted from the *in vitro* transcription experiments (Fig. 4).

An inverted repeat sequence located in the 3′ flanking region may be a terminator because such a sequence can be found in the transcriptional terminator regions in *E. coli* (20). This presumptive terminator in the 3′ flanking region of *spo0F* gene is only 680 bases away from the putative promoter, whereas the 850-base RNA was considered to be the *spo0F* transcript made *in vitro*. One possible explanation of this discrepancy could be that the *spo0F* transcript made *in vitro* was not properly terminated and resulted in the "run-off" product, due to the salt concentrations used in the *in vitro* reactions. The distance between the putative promoter and the 3′ end of 0F fragment was estimated to be about 850 bases which corresponded to the length of "run-off" transcript (Fig. 2).

It has been reported that the 3′ end of 16S rRNA forms a base-paired complex with a sequence preceding the initiation codon (21). In the *spo0F* gene, a 5′ A-A-A-G-G-A-G-G 3′ sequence situated 8 bp prior to the initiation codon (Fig. 4) is perfectly complementary with an octanucleotide sequence on 3′ end of 16S rRNA (discussed below). The distance between the ribosomal binding site and the initiation codon is close to the average distance found in other ribosome binding sites (21).

Analysis of the Gene Products. Expression of the *spo0F* gene in pUBSF13 was detected by the maxicell method (22). Protein synthesis in maxicells was carried out in the presence of [^{14}C]leucine. The protein synthesized with cell lysates was analyzed by NaDodSO$_4$/polyacrylamide gel electrophoresis. The results of these experiments suggested *in vivo* synthesis of a 20-kilodalton protein by pUBSF13 harboring the 0F fragment (Fig. 5). A derivative plasmid pUBSFΔB which lacked a part

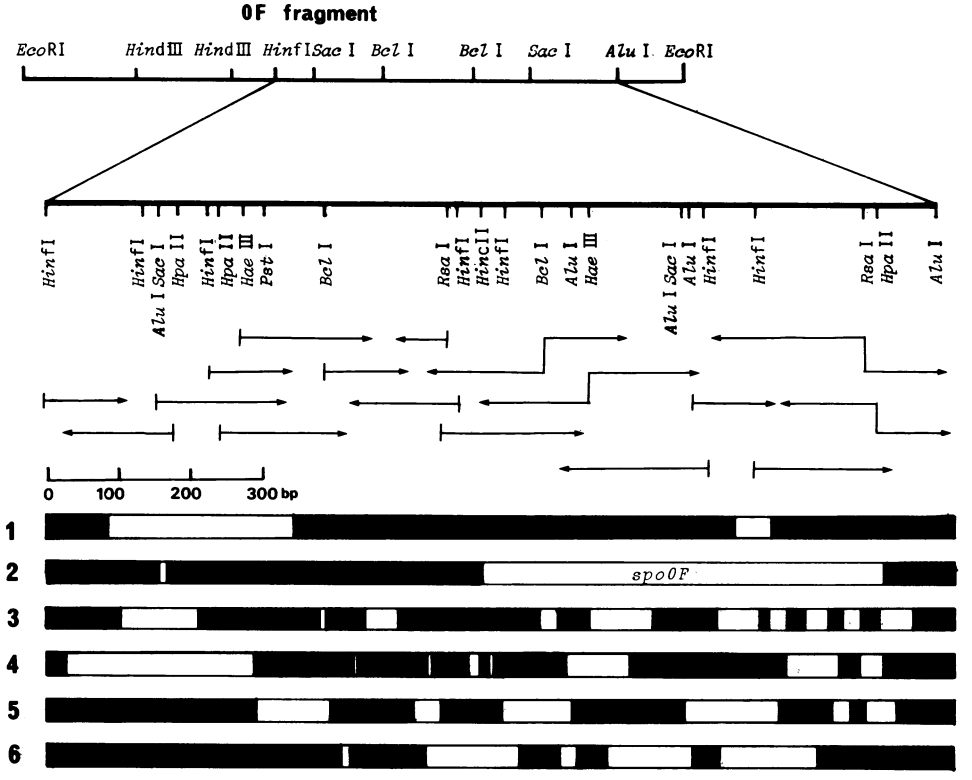

FIG. 3. Restriction endonuclease maps and sequence determination experiments. (*Upper*) Detailed physical map in the *Hin*fI–*Alu*I region of the 0F fragment. Each arrow represents a single Maxam–Gilbert sequence determination experiment. (*Lower*) Coding potential of this DNA in all six reading frames: □, open reading frame region; ■, noncoding region. The coding frame for the *spo0F* gene was determined to be frame 2, as described in the text.

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                                                                                                    80
GTCAGTTAGA CTTCAGGGGC AGATATTTTT TGACGGCGTC TCTGATTTCG TCGATGTCAA ACGGCTTGGC AAAGTGCGTC

                                                  SacI                                              160
AGAGCGCCCA ATTCCTTCGA TTCCTGGATC ATGTCGAGCT CTCCGTATGC CGTCATGATA ATGACCCGGA TGTTTTCGTC

                                                                                                    240
AATGACCTTC ATCCGTTTTA AGATTTCGAT TCCGTCCATG CCGGGAATTT TCATGTCCAA CAGCACAAGG TCGGGCCGTT

                                  PstI                                                              320
CTTTTGTCAC AATGTCAAGC GCCTGCAGGC GTTCGCAGCC TGAAACGTCT GGTAGCCTTC TTTATTGAAC ACTTCATTTA

                                  BclI                                                              400
GCAAAATACG AATGCCGTAT TGATCATCAA CGATTAAAAT TTTTTCATTC ATCATTTTAC ACCCCAATAT TATGATTTTC

                                                                                                    480
GTCAAAAGTA AGCAGTATTG TATGTATTCT GTTTGATTTT CCTATTTCCT TTAATTATAA TAGTCTACTT TACGACATTT

                                                                                                    555
TCTGAGCATT TTCTCTTTTG TTGTATACTG ATATTGTACG TTATAAAGGA GGATTCACTT   ATG TTG AAA ATA TTC
                                                                   Met Leu Lys Ile Phe
                                                                                                    621
ACG ACG CAG TTA ACA GGT ATT TTT TCC CGC ATT CAG GAT AAG GAA TCT GAC GCG ATT GAA GAT GGG
Thr Thr Gln Leu Thr Gly Ile Phe Ser Arg Ile Gln Asp Lys Glu Ser Asp Ala Ile Glu Asp Gly
                                  BclI                                                              687
GCG CGG CTG CTT GCT CAA GCG GTG ATC AGC GGG CAT TCC ATT TAT TTA TAC GGA GCG AAT GAG CTT
Ala Arg Leu Leu Ala Gln Ala Val Ile Ser Gly His Ser Ile Tyr Leu Tyr Gly Ala Asn Glu Leu
                                                                                                    753
CAG GGC GTC TTT TAT GAG GCC ACC GAA AGC AAA GAA CCC TTC CCA TCT GTC AAA GCC TTT CCA GAA
Gln Gly Val Phe Tyr Glu Ala Thr Glu Ser Lys Glu Pro Phe Pro Ser Val Lys Ala Phe Pro Glu
                                                                                                    819
AAC GCT GAG GAA GTG ACA GAA AGC GAC AGG GTG CTG ATG TTT TGC TCA GGG ACG GGC ACA GCC GAA
Asn Ala Glu Glu Val Thr Glu Ser Asp Arg Val Leu Met Phe Cys Ser Gly Thr Gly Thr Ala Glu
          SacI                                                                                      885
GAA CAG GAG CTC GCA AAA GAG CTT TAT GAA AAA GGT GCG GGA GTC GTA TGC GTA TCG CCC GCA GCC
Glu Gln Glu Leu Ala Lys Glu Leu Tyr Glu Lys Gly Ala Gly Val Val Cys Val Ser Pro Ala Ala
                                                                                                    951
AAA GAC AGT GCG GGA ATA GAA CAG TAT TGT GAT GTG CAT ATT GAT TCT AAA TTA AAA ATG CCG CTT
Lys Asp Ser Ala Gly Ile Glu Gln Tyr Cys Asp Val His Ile Asp Ser Lys Leu Lys Met Pro Leu
                                                                                                   1017
GTT CCC GAT GAA GAC GGC ACC CGT TAC GGG TTT CCC TCT TTA ATG ACA GCA CTG TAT GTC TAT CAC
Val Pro Asp Glu Asp Gly Thr Arg Tyr Gly Phe Pro Ser Leu Met Thr Ala Leu Tyr Val Tyr His
                                                                                                   1082
GCT TTA TCG TTT ACA CTA AAA GAA ATT CTG CAA GAG TAT GCA TAA       TATCTTATTG TACATGCTGG
Ala Leu Ser Phe Thr Leu Lys Glu Ile Leu Gln Glu Tyr Ala ***
                                                                                                   1162
AACTTGCCGG AAACAAATAA AAAAGACTTG CCCGCTTTTG ACAAACGGCA AGTCTTTTTT ATTACTTCTG ATTTGCAGCT
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FIG. 4. Nucleotide sequence of the *spo0F* gene and the adjacent region. Only the sequence confirmed by two independent experiments is shown. The inferred amino acid sequence of *spo0F* is also shown. The presumptive "−35 sequence", Pribnow sequence, and Shine–Dalgarno ribosome binding sequence are enclosed in boxes. The putative terminator and 5′ T-A-T-T-G-T 3′ sequence discussed in the text are underlined.

of the structural gene of *spo0F* lost the ability to synthesize the protein. The molecular weight of this protein was in agreement with the value calculated on the basis of the predicted amino acid composition, 19,065. Another protein, of molecular weight about 29,000, found in the same experiment was thought to be the product of a 1,000-base transcript of 0F fragment because this protein was synthesized by pUSFΔB but not by pUB110 (Fig. 5).

DISCUSSION

Reports of nucleotide sequences of *B. subtilis* chromosomal genes have been scarce. We have determined the nucleotide sequence of the *spo0F* gene region. The *spo0F* gene is one of the *spo0* type genes which are known to control the initial events of spore formation. The nucleotide sequence was found to contain only one long open reading frame consisting of 173 codons, and the presumptive molecular weight of the gene product was calculated to be 19,065. Expression of the *spo0F* gene of pUBSF13 was examined by the maxicell method, and a 20-kilodalton protein was detected by $NaDodSO_4$/polyacrylamide gel electrophoresis (Fig. 5). Direct correlation of the *spo0F* gene with the 20-kilodalton protein was proved because pUBSFΔB lacking a part of the *spo0F* cistron did not cause the protein synthesis. Many papers have dealt with sporulation genes but its gene product has not been identified at the molecular level.

The maxicell method used for identifying the *spo0F* gene product originally was developed with *E. coli* CSR603 (*recA1*, *uvrA6*, *phr-1*) by Sancar *et al.* (22). We adapted this method for *B. subtilis* and obtained successful results with strain UOT0277 (*recE4*, *hisA1*, *metB5*, *nonB1*). This method was found to be useful for identifying proteins encoded by plasmid DNA in *B. subtilis*.

σ55 is a major component of RNA polymerase in the actively growing cells of *B. subtilis*, and it has been reported that σ55 RNA polymerase recognizes the promoter sequence similar to the *E. coli* consensus sequence (24). Recently, it has been shown that minor transcriptional determinants σ37, σ29, and σ28 also exist in growing and sporulating cells (25–28).

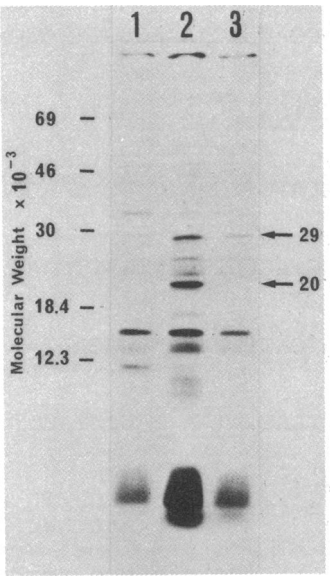

FIG. 5. Polypeptides synthesized by plasmids pUB110, pUBSF13, and pUBSFΔB. Maxicells carrying plasmids were incubated with [^{14}C]leucine. Lysates of these maxicells were electrophoresed on 15% NaDodSO$_4$/polyacrylamide gel (23). Lanes: 1, pUB110; 2, pUBSF13; 3, pUBSFΔB. [^{14}C]Methyl-labeled cytochrome *c* (12,300 daltons), lactoglobulin A (18,367 daltons), carbonic anhydrase (30,000 daltons), ovalbumin (46,000 daltons), and bovine serum albumin (69,000 daltons) were used as molecular weight standards. Minor bands (other than the 29- and 20-kilodalton ones) were found in all lanes of the original photograph.

Losick and Pero (19) suggested that the divergent promoter sequences were recognized by individual σ subunits in RNA polymerases of *B. subtilis*. A characteristic heptamer sequence 5′ T-A-T-T-G-T-T 3′ was found in the promoter region of *spoVC* gene (29) which was known to be transcribed *in vitro* by σ 37 RNA polymerase. Moran *et al.* reported that the two promoters for the 0.4-kb (*spoVG*) gene, which is catalyzed *in vitro* by a σ 37 RNA polymerase, retain the similar sequences 5′ A-A-T-T-G-A-T 3′ and 5′ T-A-A-T-G-C-T 3′ about 10 bp upstream from the two start points of *in vitro* transcription (30). The promoter region of *spo0F* gene also contains the 5′ T-A-T-T-G-T-A 3′ heptamer (Fig. 4), although it is not yet known whether the *spo0F* gene is transcribed *in vitro* by σ 37 RNA polymerase or not.

Another interesting finding in *spo0F* was concerned with translation. It has been shown that the sequences of the ribosome binding sites for eight genes of Gram-positive sources are capable of forming stable complexes with the 3′ end of *B. subtilis* 16S rRNA (31). McLaughlin *et al.* (32) have suggested that mRNAs from Gram-positive organisms have stronger Shine–Dalgarno complementality with 16S rRNA than do those of *E. coli*.

In the *spo0F* gene, a 5′ A-A-A-G-G-A-G-G 3′ sequence is situated prior to the initiation codon. This polypurine region can pair with eight nucleotides on the 3′ end of *B. subtilis* 16S rRNA (C. Woese, cited in ref. 31) as follows:

mRNA 5′ ..... AAAGGAGG .. 3′

rRNA 3′ (HO)UCUUUCCUCC .. 5′.

Based on the rules of Tinoco *et al.* (33) and Borer *et al.* (34), the calculated ΔG for this interaction is −18.3 kcal/mol (1 cal = 4.184 J). Therefore, this sequence seems capable of forming a strikingly stable complex with the 3′ end of 16S rRNA. These results offer an additional evidence to the proposal by Rabinowitz's group (31, 32, 35) that an extensive mRNA–rRNA interaction is a requirement for efficient translation by *B. subtilis* ribosomes.

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