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Isolation of the Second *Bacillus thuringiensis* RNA Polymerase That Transcribes from a Crystal Protein Gene Promoter

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A crystal protein gene of *Bacillus thuringiensis* subsp. *kurstaki* HD-1-Dipel is transcribed in vivo from two overlapping promoters that are activated at different times during sporulation. We reported earlier (K. L. Brown and H. R. Whiteley, Proc. Natl. Acad. Sci. USA 85:4166–4170, 1988) that an RNA polymerase containing a σ subunit with an apparent M_r of 35,000 can transcribe in vitro from the promoter utilized from early to midsporulation. We now report the isolation of an RNA polymerase containing a σ subunit with an M_r of ca. 28,000; this polymerase activates transcription in vitro from the promoter used from mid- to late sporulation. This form of RNA polymerase also directs transcription in vitro from promoters preceding two other crystal protein genes and a gene coding for a spore coat protein. On the basis of a comparison of the four promoters, we propose the following consensus sequence for the −10 region recognized by RNA polymerase containing the M_r-28,000 σ subunit: 5′-TNATANNaTGag-3′. No consensus sequence could be derived for the −35 region. When the N-terminal amino acid sequence of the σ^{28} polypeptide was aligned with the amino acid sequences of known σ subunits, significant homology was found with the N terminus of the mature form of the σ^{K} subunit of RNA polymerase isolated from sporulating cells of *Bacillus subtilis*.

Bacillus thuringiensis synthesizes large amounts of insecticidal proteins that accumulate to form crystals in the cytoplasm. Because crystal protein genes are expressed only during sporulation and are regulated primarily at the level of transcription (41), they provide excellent markers for study of the transcriptional mechanisms that control sporulation.

Sporulation as a developmental paradigm has been elucidated to some extent in *Bacillus subtilis* (reviewed in references 18 and 19). A hallmark of bacterial sporulation is the asymmetric septation that divides the cell into two unequal compartments having markedly different developmental fates. These separate developmental fates come about through a series of sequential steps initiated within each compartment. Temporal control of these developmental processes is achieved at least in part at the level of transcription initiation by the synthesis of σ subunits that function exclusively during sporulation. Several σ subunits have been found in association with the core subunits of *B. subtilis* RNA polymerase (reviewed in references 9, 11, 18, 19, and 24); to date, four are known to be sporulation specific. Two sigma factors, σ^{E} and σ^{F}, have been shown to be present during the formation of the asymmetric septum (24); σ^{E} is thought to be present in both the forespore and mother cell compartments later in sporulation (6). Two σ subunits have been identified that are compartment specific: the σ^{K} polypeptide, which functions exclusively in the mother cell (15, 33), and σ^{G}, a forespore-specific σ subunit (34).

Genes encoding the CryIA class of crystal proteins have promoter regions consisting of two overlapping promoters, Bt I and Bt II (reviewed in references 17 and 40). This was first observed for the gene encoding the 133-kDa toxin cloned from *B. thuringiensis* subsp. *kurstaki* HD-1-Dipel (41), now designated *cryIA(a)* (12). We previously reported (5) the isolation of a form of RNA polymerase, $E\sigma^{35}$, that directs in vitro transcription from Bt I; in vivo, this promoter is utilized during early to midsporulation.

In this paper we describe the isolation and specificity of another form of RNA polymerase from *B. thuringiensis* subsp. *kurstaki* HD-1-Dipel. This RNA polymerase is present during mid- to late sporulation (i.e., at a time when Bt II is used in vivo); it contains a σ subunit having an apparent M_r of 28,000 and drives transcription in vitro from the Bt II promoter. $E\sigma^{28}$ also transcribes from promoters for two additional crystal protein genes, *cryIB* and *cytA*, and from a promoter for *cotT*, a spore coat gene. On the basis of comparison of the DNA sequences of the promoter regions for these four genes, we propose the following preliminary consensus −10 recognition sequence for RNA polymerase containing the σ^{28} polypeptide: 5′-TNATANNaTGag-3′. Comparison of the N-terminal amino acid sequence of the σ^{28} polypeptide with the deduced amino acid sequence of the mature form of the σ^{K} polypeptide of *B. subtilis* (33) indicates that the sequences of these two proteins are nearly identical for the first 18 residues.

MATERIALS AND METHODS

Purification of RNA polymerase. *B. thuringiensis* subsp. *kurstaki* HD-1-Dipel (a derivative of strain HD-1 [14]) was grown to stage IV to V of sporulation, when phase light-gray prespores appear; *B. thuringiensis* at this stage of sporulation shows a high level of transcription from the Bt I and Bt II promoters in vivo (41). Cultures were grown in M/2–0.1% glucose medium and synchronized as described previously (41); sporulation was monitored with phase microscopy. Approximately 100 g of cells was disrupted and subjected to the purification steps described previously for the $E\sigma^{35}$ enzyme (5); fractions from the Bio-Gel A 0.5m column containing transcriptional activity were loaded onto a DNA-cellulose column and eluted with a linear gradient of 0.35 to 1.0 M NaCl in 20 mM Tris buffer, pH 7.9. Two partially overlapping enzyme activities were detected in fractions from the DNA-cellulose column: (i) fractions that contained Bt II-specific transcribing activity and began to elute at 0.44 M NaCl and (ii) fractions that had Bt I-specific transcribing activity and began to elute at 0.48 M NaCl. It should be

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noted that in cells harvested at stage III to IV of sporulation, Bt II-specific activity was not detected and $E\sigma^{35}$ was eluted from DNA-cellulose at 0.6 to 0.8 M NaCl (5). The DNA-cellulose fractions capable of transcribing from Bt II were pooled and loaded onto a BioRex-70 column (20), and proteins were eluted by a linear gradient from 0.1 to 0.6 M NaCl in 10 mM Tris buffer, pH 7.9. The Bt I-specific activity began to elute at 0.24 M NaCl, and the Bt II-specific activity began to elute at 0.28 M NaCl. Previously described methods (5) were used to obtain preparations of RNA polymerase from exponentially growing *B. thuringiensis* and *B. subtilis* and for the analysis of the protein compositions of various fractions. Protein composition was monitored by sodium dodecyl sulfate-polyacrylamide gel electrophoresis followed by staining either with Coomassie brilliant blue or with silver (26, 42).

Reconstitution of Bt II-specific transcribing activity. A fraction from the BioRex-70 column that displayed good activity from Bt II was electrophoresed on a preparative 10% polyacrylamide gel. Regions of the gel of interest were excised, and material was eluted and renatured from these gel slices (2 to 3 mm in width) as described by Hager and Burgess (10). The eluted materials were dialyzed against buffer containing 30% glycerol, 20 mM Tris (pH 7.9), and 0.1 mM EDTA. The σ subunits were removed from RNA polymerase preparations isolated from vegetatively growing *B. thuringiensis* or *B. subtilis*; the resulting core-δ (13, 31) preparations were added to renatured materials from gels and assayed for specific transcriptional activities as described previously (5).

In vitro transcription. The RNA polymerase activity of holoenzymes from vegetative cells and of fractions obtained from sporulating *B. thuringiensis* prior to DNA-cellulose chromatography were assayed by following the incorporation of [^{3}H]UTP into trichloroacetic acid-precipitable material, using *B. subtilis* phage SP82 DNA as the template (5). Fractions from sporulating *B. thuringiensis* that were eluted from the DNA-cellulose and BioRex-70 columns were analyzed by runoff transcription from templates containing the Bt I and Bt II promoters as described previously (5) except that the NaCl concentration was changed to 100 mM. The sizes of runoff transcripts were estimated by polyacrylamide gel electrophoresis under the conditions reported earlier (5).

DNA templates. Transcription from the Bt I and Bt II promoters of *cryIA(a)* (41) was monitored with linearized pKLB1 DNA as the template. This plasmid contains a DNA fragment bearing the *cryIA(a)* promoter region and a small portion of the coding sequence (5). pHES57 contains the promoter region for the gene encoding the 27-kDa crystal protein from *B. thuringiensis* subsp. *israelensis* (23) now designated *cytA* (12); the construction of this plasmid has also been described in detail elsewhere (5). Plasmid p218 (B. L. Brizzard, H. E. Schnepf, J. W. Kronstad, and H. R. Whiteley, submitted for publication) consists of a 2.5-kb DNA fragment containing the promoter region and 1,652 bp of the coding sequence of a lepidopteran-specific crystal protein gene, *cryIB* (4), cloned into pUC118 (36). The *cotT* DNA template was made by polymerase chain reaction amplification of the gene promoter from *B. subtilis*. One of the primers was identical to bases 759 through 736 of the region upstream of the coding region for *cotT* (2); this primer also had a 5′ *Eco*RI restriction site preceded by a four-base overhang (CACA) to ensure efficient cutting by the restriction enzyme. The other primer was complementary to DNA between positions 392 and 372 and had a 5′ *Bam*HI restriction site preceded by a four-base overhang (CACA), again to ensure efficient digestion by the restriction enzyme. The resulting DNA (407 bases) was analyzed by electrophoresis on 1.5% agarose gels for size and purity. The DNA fragment was then subjected to digestion with the restriction endonucleases *Eco*RI and *Bam*HI and ligated to *Eco*RI- and *Bam*HI-digested pUC118 (36), generating pKLB2. Transcription from the presumed start site for the *cotT* gene by using *Hin*dIII-linearized pKLB2 as the template would result in a runoff transcript of approximately 250 bases in length.

Transcription of the *ctc* and *spoVG* genes from *B. subtilis* was assayed by using plasmids pUC8-31 (35) and pCB1291 (25) linearized by digestion with restriction endonucleases *Hin*dIII and *Eco*RI, respectively. Transcription from the *ctc* promoter will result in a runoff transcript of 95 bases in length, and transcription initiating at the P1 or P2 promoter of the *spoVG* gene will result in a runoff transcript of either 120 or 110 bases, respectively. Transcription from the promoter for the *0.3 kb* gene of *B. subtilis* was assayed by using the 290-bp *Eco*RI-*Hin*dIII fragment from plasmid pMS2 (32); transcription from the *0.3 kb* gene promoter to the *Hin*dIII site will result in a runoff transcript of 174 bases in length.

Transcription mapping. The 5′ termini of RNAs synthesized in vivo and in vitro were determined by using oligonucleotide primers complementary to nucleotides in the following regions of the genes: for *cryIA(a)*, nucleotides 538 to 552 (30); for *cytA*, nucleotides 46 to 61 (1; this corresponds to nucleotides 554 to 569 in Fig. 2 of reference 38); for *cotT* (2), nucleotides 392 to 372; and for *cryIB*, nucleotides −71 to −85 upstream from the translational start site (Brizzard et al., submitted). The oligonucleotides were 5′-end labeled by using [γ-^{32}P]ATP and polynucleotide kinase, annealed (67°C for 5 min followed by incubation at 37°C for 15 min) to RNAs, and extended with Moloney murine leukemia virus reverse transcriptase by using the reaction conditions recommended by the manufacturer (Bethesda Research Laboratories). RNA synthesized in vivo was isolated from sporulating *B. thuringiensis* as described previously (5). Base-specific markers for determining the lengths of the extended primers were generated by sequencing the promoter regions by the dideoxy method (29) using the oligonucleotides described above as primers. The DNA-sequencing reactions and the extended primers were analyzed by electrophoresis on 6 or 8% polyacrylamide–7 M urea sequencing gels buffered with Tris-borate-EDTA.

N-terminal sequence analysis of the σ^{28} polypeptide. A fraction from the BioRex-70 column containing $E\sigma^{28}$ was subjected to electrophoresis on a 10% polyacrylamide gel and transferred onto an Immobilon-P membrane (Millipore); electroblotting of the proteins onto the membrane and subsequent treatment of the membrane prior to gas-phase sequencing was carried out as described by Matsudaira (22).

Miscellaneous methods. Protein concentrations were determined by the method of Bradford (3), using bovine immunoglobulin as a standard; the isolation of plasmid DNA and its digestion with restriction endonucleases were accomplished by using published methods (21).

RESULTS AND DISCUSSION

Isolation of an RNA polymerase capable of directing transcription in vitro from a crystal protein gene promoter activated during middle to late sporulation. RNA polymerase isolated from vegetatively growing *B. thuringiensis* does not direct transcription from either the Bt I or the Bt II promoter (data not shown). RNA polymerase prepared from *B. thuringiensis* subsp. *kurstaki* HD-1-Dipel harvested at stage IV

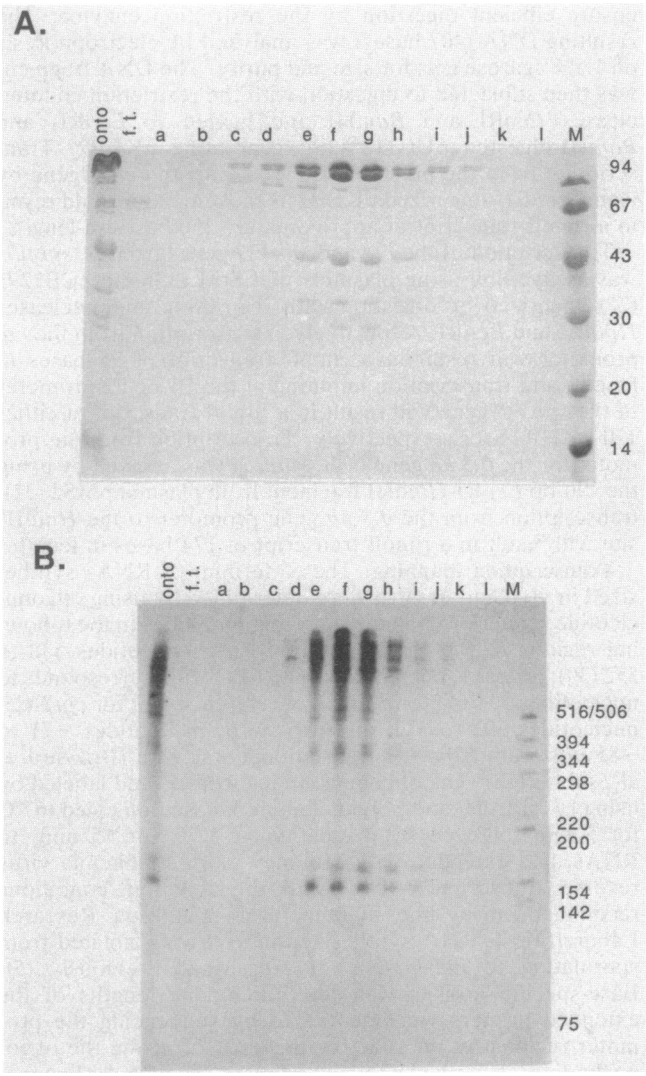

FIG. 1. Polypeptide composition and in vitro transcriptional activity of *B. thuringiensis* RNA polymerase eluted from a BioRex-70 column. (A) Photograph of a sodium dodecyl sulfate-polyacrylamide gel stained with Coomassie brilliant blue displaying protein content of fractions from BioRex-70 column. Lane M, molecular mass markers with sizes indicated in kilodaltons (in descending order: phosphorylase *b*, bovine serum albumin, ovalbumin, carbonic anhydrase, soybean trypsin inhibitor, and α-lactalbumin). (B) Autoradiogram of RNA gel displaying runoff transcripts produced by in vitro transcription from the Bt I and Bt II promoters of *cryIA(a)*, using pKLB1 linearized by *Hin*dIII digestion as the template. Lane M, 1-kb ladder, with sizes indicated in bases.

to V of sporulation (see Materials and Methods) displayed significant transcription from both the Bt I and Bt II promoters of the *cryIA(a)* gene. Figure 1 displays the polypeptide composition and the transcriptional specificity of fractions obtained from the final step of purification, chromatography on a BioRex-70 column. Analysis of the polypeptide composition demonstrated that the fractions shown in lanes e to h of Fig. 1A contained the core components of RNA polymerase (β = 145 kDa, β′ = 130 kDa, α_2 = 44 kDa), a peptide with an M_r of ca. 90,000 (probably a degradation product of the β and β′ subunits), traces of peptides with M_rs of <14,000 (possibly also degradation products), and three polypeptides with apparent M_rs of 36,000, 28,000 and 26,000. Fractions containing the latter three polypeptides yielded two short transcripts (Fig. 1B, lanes e to i) whose lengths are in good agreement with the lengths predicted for transcription from Bt I (143 bases in length) and Bt II (159 bases in length) when pKLB1 DNA has been linearized by digestion with *Hin*dIII.

Reconstitution of the Bt II-specific transcribing activity. A fraction of RNA polymerase capable of transcribing from Bt II (Fig. 1A, lane h) was subjected to preparative polyacrylamide gel electrophoresis; sections of the gel containing the 90-, 36-, 28-, 26-, and 14-kDa polypeptides were excised, and the polypeptides in the gel sections were eluted and renatured. The renatured materials were added to core-δ preparations of RNA polymerase isolated from vegetative cells of *B. thuringiensis*. A fraction containing RNA polymerase in association with the 36-, 28-, and 26-kDa polypeptides transcribed from both the Bt I and the Bt II promoters (Fig. 2A, lanes a and c). When the reaction mixtures contained core RNA polymerase plus the eluted and renatured 28-kDa polypeptide (Fig. 2A, lanes b and d), only RNAs indicative of transcription from Bt II were found.

No transcripts could be detected when core RNA polymerase was combined with eluted and renatured 26-kDa polypeptide (Fig. 2A, lane f) or with material from the area of the gel between the 26- and 28-kDa polypeptides (Fig. 2A, lane e) or when core RNA polymerase was assayed in the absence of any added materials (Fig. 2A, lane g). When materials eluted from above, at, and below the 28-kDa band (Fig. 2B) were added to core-δ RNA polymerase from vegetatively growing *B. thuringiensis*, the only transcriptional activity detected was from the Bt II promoter in the presence of the 28-kDa polypeptide. The eluted and renatured fraction that allowed transcription from Bt II did contain a polypeptide with an apparent M_r of ca. 28,000 (Fig. 2C). These observations suggest that the 28-kDa polypeptide alone is sufficient to direct transcription from the Bt II promoter when in association with core RNA polymerase—i.e., that this polypeptide is functioning as a σ subunit in vitro. Genetic studies will be needed to confirm the role of the 28-kDa polypeptide in Bt II transcription in vivo. Addition of the gel-purified σ^{28} to core-δ RNA polymerase isolated from vegetatively growing *B. subtilis* also yielded the Bt II-specific transcribing activity (data not shown), whereas addition of the ca. 90-kDa peptide, the 36-kDa polypeptide, or the <14-kDa peptides to core RNA polymerase from *B. thuringiensis* did not result in transcription from either the Bt I or Bt II promoter (data not shown). As expected from the sequences of these promoters or the compartmental specificities of their expression, the reconstituted $E\sigma^{28}$ did not transcribe from three previously characterized *B. subtilis* promoters known to require minor σ subunits. The reconstituted enzyme did not transcribe from the *0.3 kb* promoter (activated only in the forespore [27]), the *ctc* promoter (dependent on σ^B [35]), or the *spoVG* promoter (dependent on σ^H [7]) (Fig. 2A, lanes i to k).

Transcription from the Bt I promoter. Fractions containing the core RNA polymerase subunits in association with the three polypeptides of 36, 28, and 26 kDa yielded RNAs originating from Bt I and Bt II in our in vitro transcription assays (Fig. 1). The reconstitution experiments presented in Fig. 2 demonstrated that the 28-kDa polypeptide is responsible for transcription initiating at Bt II; however, when progressively larger amounts of the eluted and renatured 28-kDa polypeptide were added to core RNA polymerase preparations, weak transcription from the Bt I promoter became detectable (Fig. 3, lane e). Comparison of the Bt I

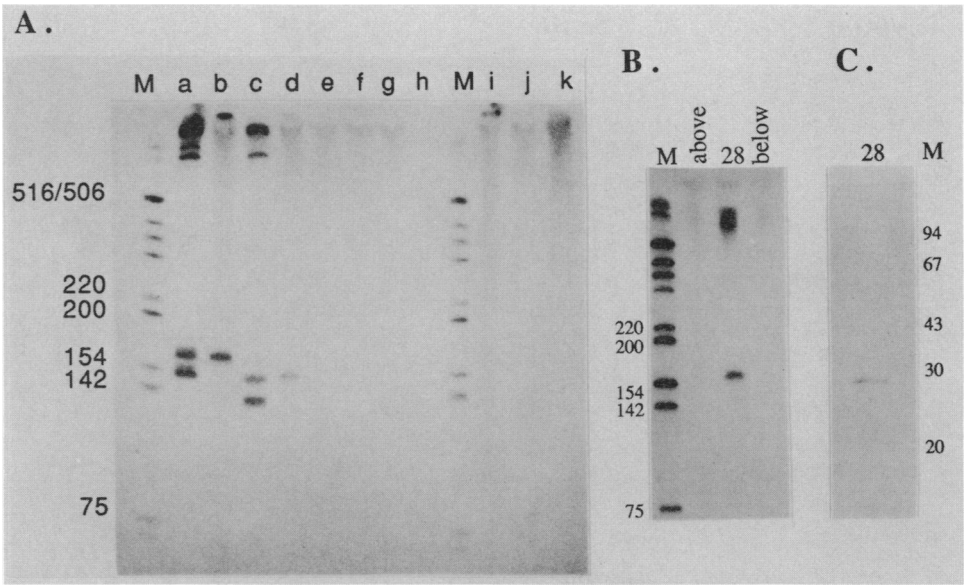

FIG. 2. Reconstitution of in vitro Bt II-specific transcribing activity. (A) Autoradiogram of RNA gel displaying runoff transcripts produced in vitro, utilizing templates containing promoters for the *cryIA(a)* gene of *B. thuringiensis* or for the *B. subtilis 0.3 kb*, *ctc*, or *spoVG* gene. Lanes a and c, RNA polymerase holoenzyme containing both Bt I- and Bt-II-specific activities; lane a, pKLB1 digested with *Hin*dIII; lane c, pKLB1 digested with *Sal*I. Lanes b and d, core RNA polymerase containing the δ polypeptide isolated from vegetatively growing *B. thuringiensis* plus added eluted and renatured 28-kDa polypeptide; lane b, pKLB1 linearized by *Hin*dIII digestion; lane d, pKLB1 linearized by *Sal*I digestion. Lanes e, f, and g, core RNA polymerase containing δ plus (i) material between the 28- and 26-kDa polypeptides (lane e), (ii) eluted and renatured 26-kDa polypeptide (lane f), or (iii) 30% glycerol dialysis buffer (lane g); pKLB1 digested with *Hin*dIII was used as the template in reactions shown in lanes e, f, and g. Lane h, empty. Lanes i, j, and k, core RNA polymerase containing δ plus added eluted and renatured 28-kDa polypeptide; lane i, template containing *0.3 kb* promoter; lane j, template containing *ctc* promoter; lane k, template containing the *spoVG* promoter. Lane M, 1-kb ladder DNA fragments, with sizes indicated in bases. (B) Autoradiogram of RNA gel showing transcripts produced with pKLB1 linearized by *Hin*dIII digestion as the template. Lanes contained core-δ RNA polymerase from vegetatively growing *B. thuringiensis* plus added eluted and renatured material from the area above the 28-kDa band, the area corresponding to the 28-kDa band, and the area below the 28-kDa band. Lane M, 1-kb ladder DNA fragments, with sizes indicated in bases. (C) Photograph of sodium dodecyl sulfate-polyacrylamide gel after electrophoresis of the preparation of eluted and renatured 28-kDa polypeptide stained with silver. Molecular mass markers with sizes in kilodaltons are indicated (as in Fig. 1A).

and Bt II promoters shows that they share certain bases in common in the −10 regions (the identical bases are 5′-CATANNNT-3′). Genetic studies will be needed to assess the significance of this observation with regard to transcription of crystal protein genes in vivo.

Comparison of the 5′ termini of RNAs produced by in vitro transcription by Eσ^{28} with 5′ termini of RNAs produced in vivo. Runoff transcription analyses (data not shown) indicated that reconstituted Eσ^{28} directed transcription from two additional crystal protein gene promoters, *cryIB* (a gene coding for a lepidopteran-specific protein cloned from *B. thuringiensis* subsp. *thuringiensis* [4]) and *cytA* (a gene coding for a 27-kDa peptide cloned from *B. thuringiensis* subsp. *israelensis* [23, 37]), as well as from *cotT* (a gene coding for a spore coat protein [2]). The 5′ termini of RNAs synthesized by reconstituted Eσ^{28} in vitro from these promoters were compared to the 5′ termini of RNAs isolated from sporulating cells of *B. thuringiensis* by primer extension with reverse transcriptase. Panel "*cryIA(a)*" of Fig. 4 demonstrates that RNAs synthesized in vitro from the Bt II promoter by reconstituted Eσ^{28} (indicated by an arrow) had 5′ termini corresponding to two of the three start points observed in vivo. As demonstrated elsewhere (Brizzard et al., submitted), *cryIB* is transcribed in vivo from two temporally regulated promoters (P1 and P2) whose sequences are very similar to those of Bt I and Bt II of *cryIA(a)*. Panel "*cryIB*" of Fig. 4 shows that transcription from the P2 promoter (indicated by an arrow) displayed some heteroge-

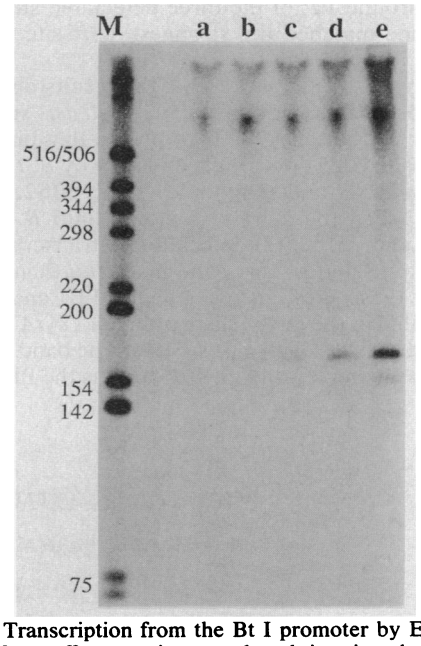

FIG. 3. Transcription from the Bt I promoter by Eσ^{28}. Autoradiogram of runoff transcripts produced in vitro by core RNA polymerase reconstituted with increasing amounts of σ^{28} with pKLB1 digested with *Hin*dIII as the template. Lanes: a, no addition; b, 3 ng of σ^{28}; c, 6 ng; d, 12 ng; e, 18 ng; M, 1-kb ladder DNA fragments, with sizes indicated in bases.

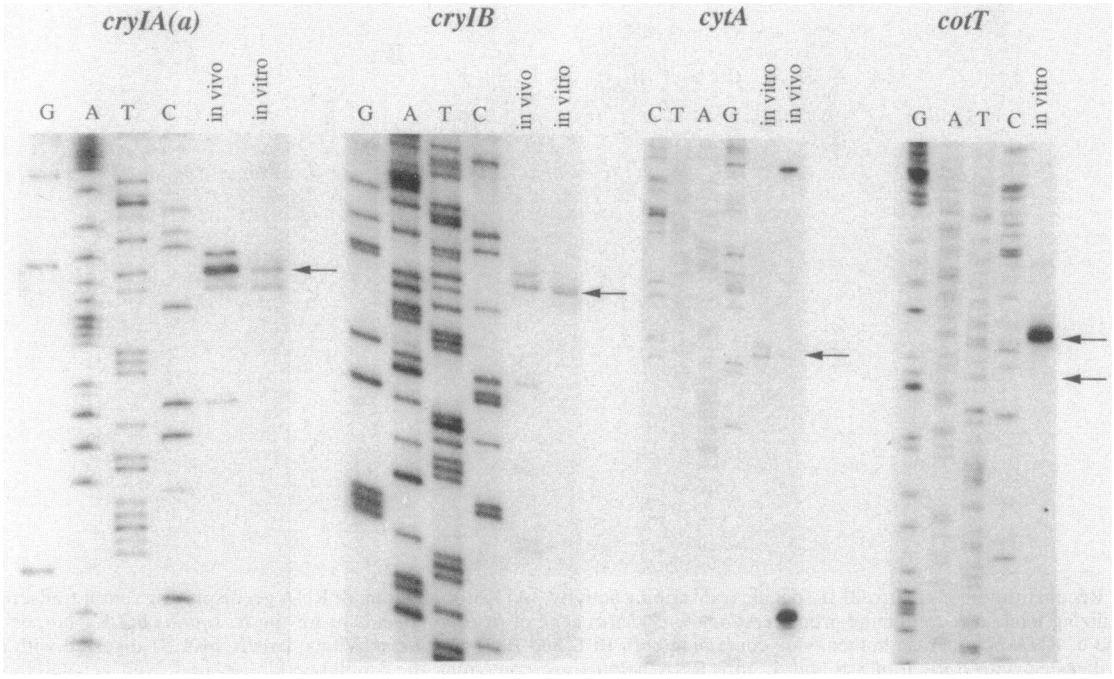

FIG. 4. Autoradiogram showing primer extension mapping of the 5′ termini of RNAs synthesized in vivo and in vitro from the *cryIA(a)*, *cryIB*, *cytA*, and *cotT* promoters. Lanes labeled G, A, T, and C show sequence analyses of the promoter regions of the respective genes; lanes labeled in vitro display 5′ termini of RNAs synthesized by reconstituted $E\sigma^{28}$; lanes labeled in vivo show 5′ termini of RNAs isolated from sporulating *B. thuringiensis*. Transcriptional start sites of interest are indicated by arrows. (The panel labeled *cytA* is in the reverse orientation of the other panels.)

neity, as in the transcription from Bt II. Two transcriptional start sites were observed in the "in vivo" lane that correspond to transcription from the P2 promoter activated late in sporulation in vivo. The "in vitro" lane also had multiple start sites around P2; of the three detectable start sites, the strongest one matched the strongest start site observed in the in vivo lane.

Panel "*cytA*" of Fig. 4 presents the results obtained from parallel experiments with this *B. thuringiensis* subsp. *israelensis* gene. Ward et al. (38, 39) reported that one transcriptional start site (PB1) was utilized in vivo by *B. thuringiensis* subsp. *israelensis* and that three (PB1, PBS2, and PBS1) were used when the gene was cloned into *B. subtilis*. In contrast, Adams et al. (1) detected two transcriptional start sites for expression of this gene in *B. thuringiensis* subsp. *israelensis*; these sites corresponded to PB1 and PBS1. The data presented in the in vivo lane of Fig. 4 "*cytA*" agree with those of Ward et al. (39) for *B. subtilis*. The band near the top of the gel in the in vivo and in vitro lanes is the PB1 start site; as reported earlier (5), $E\sigma^{35}$ directs transcription from PB1 in vitro, indicating that this is a Bt I-type promoter. The band indicated by the arrow is the start site for the PBS2 promoter, and the lower cluster of bands in the in vivo lane correspond to the start sites for the PBS1 promoter (39). As shown in the in vitro lane, the PBS2 promoter is utilized by reconstituted $E\sigma^{28}$; the band indicated by the arrow corresponds to the start site used in vivo. The low level of transcription from the PB1 promoter in vitro can be attributed to weak utilization by reconstituted $E\sigma^{28}$. The PBS1 promoter (corresponding to the band near the bottom of the gel in the in vivo lane) apparently is not utilized in vitro.

Of the crystal protein genes tested in this laboratory one, *cryIIA*, is transcribed from one promoter in vivo that is recognized by $E\sigma^{35}$ in vitro (5). Three crystal protein genes [*cryIA(a)*, *cryIB*, and *cytA*] are transcribed from multiple promoters in vivo. As stated above, the two promoters of the lepidopteran-specific genes *cryIA(a)* and *cryIB* are very similar; i.e., the downstream promoter is transcribed by

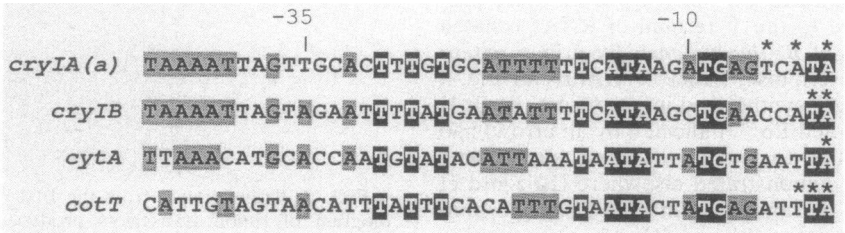

FIG. 5. Alignment of $E\sigma^{28}$ promoters. DNA sequences of the regions upstream of the transcriptional start sites for *cryIA(a)*, *cryIB*, *cytA*, and *cotT*. Bases common to all four promoters are outlined in black; bases common to three of the four promoters are shaded. Major in vivo transcriptional start sites for crystal protein gene promoters and the observed in vitro transcriptional start sites for *cotT* are indicated with asterisks.

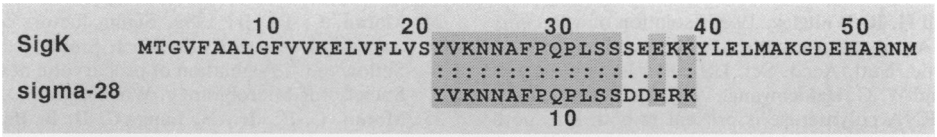

FIG. 6. Comparison of the N-terminal amino acid sequences of *B. thuringiensis* σ^{28} and *B. subtilis* σ^{K}. The first 18 residues of the σ^{28} polypeptide obtained by gas-phase sequencing are aligned with the N-terminal amino acid sequence of σ^{K} deduced from the DNA sequence (33). Conserved amino acids are indicated by double dots between the two sequences and are shaded; conservative amino acid changes are indicated by a single dot.

$E\sigma^{35}$ in vitro and is expressed during early to midsporulation in vivo, and the overlapping upstream promoter is recognized by $E\sigma^{28}$ in vitro and utilized in vivo during mid- to late sporulation. In contrast, *cytA*, which encodes a dipteran toxin, has three promoters: pBI, pBS2, and pBSI (39). The promoter transcribed by $E\sigma^{35}$in vitro (pBI) is located approximately 150 bp upstream of the promoter utilized in vitro by $E\sigma^{28}$ (pBS2); i.e., the promoters are in the order opposite from that of the promoters for two *cryI* genes. However, pBI and pBS2 are expressed during the expected stages of sporulation (transcription from pBI begins at stage II in *B. thuringiensis* and *B. subtilis*, and a low level of transcription from pBS2 first appears at stage III in *B. subtilis* [38, 39]). We have not observed transcription of the pBSI promoter in vitro by either $E\sigma^{35}$ or $E\sigma^{28}$.

Panel "*cotT*" of Fig. 4 displays the results obtained with RNA synthesized in vitro from the promoter region of the *B. subtilis cotT* gene. Our in vitro start site (upper arrow) is somewhat heterogeneous, but it differs from the previously reported (2) in vivo start site (lower arrow) by at least 10 bases. The discrepancy in results may be due to differences in methodology used to determine the start sites.

Preliminary consensus sequence for −10 region of the promoter recognized by $E\sigma^{28}$. The DNA sequences of the *cryIA(a)*, *cryIB*, *cytA*, and *cotT* promoters that are specifically transcribed by reconstituted $E\sigma^{28}$ are compared in Fig. 5. Sequence similarity in the −10 regions of these promoters and centered around nucleotide −28 is indicated. There is little sequence similarity in the −35 regions of these promoters.

It is reasonable to expect that $E\sigma^{28}$ directs RNA polymerase to promoters for genes in addition to those coding for crystal proteins. Likely candidates would be genes that are expressed during mid- to late sporulation—i.e., at the same time that transcription from Bt II is observed. RNA polymerase containing σ^{K} has been found to function at an equivalent time in the sporulation process of *B. subtilis* (15). Four *B. subtilis* genes expressed at this interval of sporulation are *cotT* (2), *cotA* (28), *gerE* (8), and *spoIVCB*, the gene encoding the amino terminus of the σ^{K} subunit (16). Alignment of the −10 regions of the promoters for the *cotA*, *gerE*, and *spoIVCB* genes with the −10 regions of the promoters for *cryIA(a)*, *cryIB*, *cytA*, and *cotT* revealed that *cotA* has four of nine bases identical with the consensus sequence for promoters recognized by $E\sigma^{28}$, whereas five of nine bases are identical in the *spoIVCB* and *gerE* promoters. It has been reported that the *spoIVCB* promoter is transcribed weakly by RNA polymerase containing σ^{K} and that transcription is enhanced by the presence of the "switch protein" encoded by *spoIIID* (15); however, RNA polymerase containing σ^{K} can efficiently transcribe from the *cotA*and *gerE* promoters in the absence of the switch protein.

N-terminal amino acid sequence analysis of the σ^{28} polypeptide. To determine the N-terminal amino acids of the *B. thuringiensis* σ^{28} polypeptide, a preparation of σ^{28} was analyzed by sequential Edman degradation as described in Materials and Methods. Comparison of this sequence with the deduced sequences of *B. subtilis* σ subunits (33) showed a strikingly similarity between the first 18 N-terminal amino acids of the *B. thuringiensis* σ^{28} subunit and the N-terminal amino acids of the mature σ^{K} polypeptide present in RNA polymerase in the mother cell compartment of sporulating *B. subtilis*. Of the 18 residues, 15 are identical; the remaining 3 amino acids are conservative changes (Fig. 6). It is likely that the σ^{28} polypeptide of *B. thuringiensis* is a homolog for σ^{K} of *B. subtilis*. Additional experimentation will be required to determine if transcription of the gene coding for the σ^{28} subunit of *B. thuringiensis* depends on $E\sigma^{28}$ and if this transcription is regulated by a homolog of the *spoIIID* gene product.

Summary. We have isolated from *B. thuringiensis* a sporulation-specific form of RNA polymerase that directs transcription from the Bt II promoter and weakly from the Bt I promoter of *cryIA(a)* in vitro. Reconstitution experiments show that a polypeptide with an apparent M_r of 28,000 functions as a σ factor; additional reconstitution experiments show that $E\sigma^{28}$ transcribes from promoters for *cryIB*, *cytA*, and *cotT*. Comparison of the N-terminal amino acid sequence of σ^{28} with that of the σ^{K} polypeptide of *B. subtilis* shows that these proteins are nearly identical at the N terminus and suggests that σ^{28} of *B. thuringiensis* is a homolog of σ^{K} of *B. subtilis*.

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