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A Polymerase Chain Reaction-Based Approach to Cloning Sigma Factors from Eubacteria and Its Application to the Isolation of a Sigma-70 Homolog from *Chlamydia trachomatis*

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Taking advantage of the known sequence conservation of portions of bacterial sigma factor proteins, we have designed degenerate oligonucleotides corresponding to these domains and used these synthetic DNA sequences as primers in a polymerase chain reaction (PCR) to amplify DNA sequences from the chlamydial genome. The PCR products were used as a probe to recover the genomic fragments from a library of cloned murine *Chlamydia trachomatis* DNA. Sequence analysis of one of these clones revealed striking homology to the sigma-70 protein of *Escherichia coli* and the sigma-43 protein of *Bacillus subtilis*, strongly implying that this locus (*sigA*) encodes the major vegetative sigma factor of murine *C. trachomatis*. This PCR-based approach will be broadly applicable to the cloning of major sigma factors from other eubacteria.

Central to transcriptional specificity in procaryotes is the interaction between RNA polymerase and the promoter sites of genes. The polymerase molecule is a multisubunit enzyme composed of α, β, β', and sigma subunits. The core enzyme, $\alpha_2\beta\beta'$, is a non-specific DNA-binding protein. Holoenzyme, formed by the association of the sigma subunit with core enzyme, has the property of sequence-specific DNA recognition, permitting the specific binding of RNA polymerase to promoter sequences (reviewed in reference 15). The major sigma subunit of *Escherichia coli* and *Salmonella* species, sigma-70, is responsible for RNA polymerase binding to the basic promoter motif (TATAAT at −10 and TTGACA at −35). The corresponding sigma factor in *Bacillus subtilis* is sigma-43.

Many procaryotes, however, employ additional sigma factors to regulate the transcription that underlies specialized cellular functions (reviewed in reference 8). For example, the gram-positive organism *B. subtilis* utilizes a sophisticated program involving as many as seven distinct sigma factors to direct the strict temporal expression of gene products during the complex process of sporulation (6, 7, 11, 16, 21–23). More recent studies have indicated that several diverse species of bacteria, including *E. coli* and other members of the family Enterobacteriaceae (12–14, 18, 25), *Streptomyces coelicolor* (4, 5, 34, 36, 37), and *Neisseria gonorrhoeae* (19), utilize multiple sigma factors to control the expression of gene products involved in the heat shock response, nitrogen fixation, and flagellar protein expression as well as other uncharacterized cellular processes (6, 8).

We are interested in understanding the life cycle and pathogenic mechanisms of *Chlamydia trachomatis*, a medically important gram-negative bacterium that causes a large array of sexually transmitted diseases. This obligate intracellular parasite of eucaryotic cells replicates via a unique developmental cycle involving the serial alternation of two distinct forms within the cytoplasm of the infected cell. The life cycle commences when the extracellular form, the sporelike, metabolically inactive elementary body, is taken up by the host eucaryotic cell. Upon binding to the host cell membrane and subsequent internalization into a host-derived endosome, the elementary body undergoes a striking morphologic transformation into the intracellular vegetative form, the reticulate body. The reticulate body replicates by binary fission approximately 100- to 1,000-fold while enclosed within this vacuole in the host cell cytoplasm. These reticulate bodies subsequently redifferentiate into elementary bodies and are released from the host cell, completing the developmental cycle. Although this life cycle has been well described morphologically, very little is known about the molecular mechanisms which underlie these events (for reviews, see references 2, 28, and 29). The chlamydial developmental cycle, which formally resembles the life cycle of the sporulating bacterium *B. subtilis* (21, 23), is presumably the end result of a temporally regulated program of gene expression.

A major focus of our research has been to elucidate the *cis* elements and *trans*-acting factors that underlie the regulation of gene expression during the chlamydial life cycle. In earlier studies, we and others have shown that chlamydial promoter sequences appear to be different from those of other procaryotes (9, 27, 32), so much so in fact that no chlamydial promoter tested so far functions properly in *E. coli* (27; J. N. Engel and D. Ganem, *Immune Recognition and Evasion: Molecular Aspects of Host-Parasite Interaction*, in press). Thus, it is likely that a fuller understanding of chlamydial gene transcription will require a more detailed characterization of chlamydial RNA polymerase. We are particularly interested in chlamydial sigma factors, both because of their role in promoter recognition generally and because of the possibility that, by analogy with the sporulating bacterium *B. subtilis*, multiple sigma factors might be involved in regulation of the chlamydial intracellular life cycle. In this article, we report the cloning and characterization of a chlamydial homolog of sigma-70, employing a strategy that makes use of the polymerase chain reaction (PCR) to directly amplify related sequences from the chlamydial genome. This approach is generally applicable to the cloning of eubacterial sigma factors.

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MATERIALS AND METHODS

Reagents. Products were obtained from the following sources and were used according to the manufacturers' specifications. Restriction enzymes, bacterial alkaline phosphatase (BAP), exonuclease III, and T4 DNA ligase were from New England BioLabs, Inc. (Beverly, Mass.); T4 polynucleotide kinase was from Boehringer Mannheim Biochemicals (Indianapolis, Ind.); DNA polymerase I was from Pharmacia Fine Chemicals (Piscataway, N.J.); ^{32}P-containing radioisotopes were from Amersham Corp. (Arlington Heights, Ill.); [^{35}S]methionine was from ICN (Irvine, Calif.); *Thermus aquaticus* DNA polymerase was from Cetus Corp. (Emeryville, Calif.); Seaplaque and Seakem agarose were from FMC Bioproducts (Rockland, Maine); and ampicillin, kanamycin, and rifampin were from Sigma Chemical Co. (St. Louis, Mo.).

Nucleic acid preparation and analysis. Chlamydial DNA from the mouse pneumonitis (MoPn) or the lymphogranuloma venereum (LGV) strain (serovar L2) of *C. trachomatis* was prepared as described previously (9). Standard recombinant DNA methods were used for nucleic acid preparation and analysis (24). Restriction fragments were subcloned into a pGEM7Zf (Promega Biotech, Madison, Wis.) plasmid vector. Southern and Northern (RNA) blotting were carried out as described previously (9). Low-stringency Southern blotting was carried out by hybridizing and washing the filters at 50 instead of 65°C. Radioactive DNA probes were labeled by nick-translation or by 5'-end labeling with T4 polynucleotide kinase (24).

Synthetic oligonucleotides. The following single-stranded oligonucleotide primers were synthesized by the Biomedical Resource Center at the University of California, San Francisco (nucleotides in parentheses indicate the bases used in the degenerate positions): primer 1, GGCTCGAGAT(TCA)GC(AGCT)AA(AG)(AC)G(AGCT)TA; primer 2, GGCTCGAGGG(AGCT)(TC)T(AGCT)ATGAA(AG)GC(AGCT)GT; and primer 3, CCGAATTCGC(TC)TG(AGCT)C(TG)(AGT)ATCCACC.

PCR. PCR was performed with a Cetus/Perkin-Elmer DNA thermocycler. Reaction mixtures (100 μl) contained 100 pmol of either primer 1 or 2 and 100 pmol of primer 3, 1 μg of DNA, all four dNTPs at 1 mM each, 50 mM KCl, 10 mM Tris chloride (pH 8.0), 2 mM $MgCl_2$, 0.01% gelatin, and 2.5 U of *Thermus aquaticus* DNA polymerase. The reaction mixture was overlaid with a drop of paraffin oil and subjected to 35 cycles consisting of a 2-min denaturation period at 94°C, a 2-min annealing period at 45°C, and a 2-min extension period at 72°C. After analysis of the PCR product on a 1.5% low-melting-point agarose gel (SeaPlaque), the amplification product was purified from the gel followed by isolation with glass beads (GeneClean; Bio101, La Jolla, Calif.). The gel-purified product was digested with *Eco*RI and *Xho*I and cloned into pGEM7Zf previously digested with *Eco*RI and *Xho*I, followed by treatment with BAP.

Preparation and screening of a chlamydial DNA library. Chlamydial DNA was digested with *Eco*RI and cloned into a pUC8 (Pharmacia, Piscataway, N.J.) vector previously cleaved with *Eco*RI and dephosphorylated with BAP. A total of 900 colonies were stabbed onto L-broth plates containing ampicillin (50 μg/ml). After overnight growth at 37°C, the plates were overlaid with Hybond filters (Amersham, Arlington Heights, Ill.). Filters bearing colonies were soaked in 0.5 M NaOH–1.5 M NaCl, followed by 0.5 M Tris chloride (pH 8.0)–1.5 M NaCl. Following UV light cross-linking, the filters were hybridized as described previously (9) to a 5'-end-labeled probe made from the PCR product (primed by oligonucleotides 1 and 3). The *Eco*RI fragments from the clones demonstrating hybridization to this probe was then recloned into the *Eco*RI site of pGEM7Zf for further analysis.

DNA sequencing. The dideoxy chain termination method of DNA sequencing (26) was carried out on double-stranded fragments cloned into pGEM7Zf with the Sequenase kit (United States Biochemical Corporation, Cleveland, Ohio). Sequencing reactions were primed with oligonucleotides homologous to the T7 and SP6 promoters (Promega Biotech, Madison, Wis.) flanking the cloned inserts in the pGEM7Zf vector. Nested deletions were generated by using exonuclease III according to the manufacturer's specifications. Clone A was completely sequenced on both strands from the *Xba*I site to the *Xho*I site (see Fig. 5B). Regions of the relevant terminal *Xho*I-*Eco*RI fragment of clone B were sequenced on one strand only.

Overproduction of bacterially encoded proteins. Overproduction of proteins encoded by cloned genes of interest was accomplished by introducing the corresponding plasmid into a strain of *E. coli* harboring pGP1-2 (33), a pGEM-compatible plasmid that encodes the phage T7 RNA polymerase under control of a thermolabile lambda repressor. The following plasmids were constructed in pGEM7Zf (see Fig. 5B): clone A (full-length sigma-A gene), p297 (*Xba*I-*Eco*RI fragment from clone A), and p335 (C-terminal *Eco*RI-*Xho*I fragment from clone A). Induction of the plasmid-encoded gene product was carried out as follows. Strains were grown at 30°C in L broth (24) containing kanamycin (50 μg/ml) and ampicillin (100 μg/ml) to an A_{600} of 0.6. Samples (1 ml) were pelleted and suspended in M9 minimum medium (24) containing thiamine and all amino acids except methionine. The cultures were then transferred to 42°C for 30 min, at which time rifampin (100 μg/ml) was added. Following further incubation at 42°C for 10 min, [^{35}S]-methionine was added (50 μCi/ml), and the cultures were grown at 37°C for 30 min. The bacteria were pelleted and lysed in 100 μl of Laemmli buffer containing 5% (vol/vol) β-mercaptoethanol (20). Electrophoresis of the protein products was carried out on 12% polyacrylamide–sodium dodecyl sulfate (SDS) slab gels (20), as modified (1).

RESULTS

Isolation of a sigma factor gene. Because chlamydiae are significantly diverged from the Enterobacteriaceae, we suspected that it would not be possible to use the cloned sigma-70 coding region from *E. coli* as a probe to isolate the corresponding gene from chlamydiae. However, amino acid sequence analysis of previously characterized sigma factor proteins has revealed that certain regions of sigma factor proteins are highly conserved. Based on these observations from multiple sequence alignments, we reasoned that chlamydial sigma factor sequences might be amenable to isolation by utilizing the PCR, with oligonucleotide primers derived from the conserved regions used to amplify the intervening regions.

Four such conserved regions have been identified (Fig. 1), and their conservation may reflect common functional attributes of the bacterial sigma factors (15). Region 1 extends from approximately amino acids 1 to 125 in the *E. coli* sigma-70 and the *B. subtilis* sigma-43 proteins; its function in holoenzyme is unclear, and it is not conserved in the minor sigma factors. Region 2, spanning approximately 70 amino acids, contains some of the most highly conserved se-

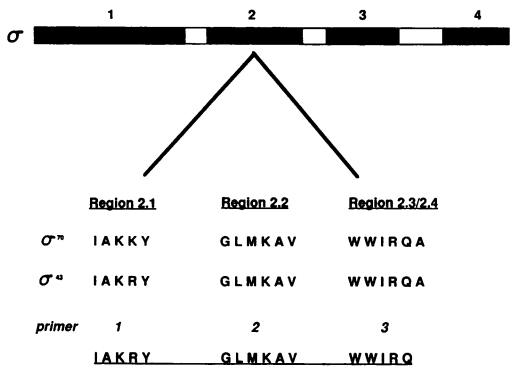

FIG. 1. Diagram of the conserved regions of sigma factors and the specific protein domains to which the degenerate oligonucleotide primers were designed. The shaded boxes represent conserved regions 1, 2, 3, and 4 of sigma factors (15). Below the diagram is an enlargement of region 2. The conserved amino acid sequences of *E. coli* sigma-70 (top line) and *B. subtilis* sigma-43 (second line) within regions 2.1, 2.2, and 2.3/2.4 from which the degenerate oligonucleotide primers were derived are shown, using the one-letter code.

quences among the sigma factors and can be divided into four subregions. Regions 2.1 and/or 2.3 are thought to be involved in DNA melting at the site of mRNA transcription initiation, while regions 2.2 and 2.4 are believed to mediate binding to core RNA polymerase enzyme and −10 recognition, respectively. Regions 3 (spanning approximately 50 amino acids) and 4 (spanning about 60 amino acids) are located in the C-terminal portion of sigma factors and have in common a potential helix-turn-helix motif. Mutational analysis suggests that region 4 is involved in promoter recognition, specifically at the −35 box (10, 31).

Degenerate oligonucleotide probes homologous to regions 2.1, 2.2, and 2.3 (Materials and Methods and Fig. 1) were synthesized; *Xho*I or *Eco*RI sites were included at the 5′ and 3′ ends, respectively, to facilitate the subsequent cloning of the PCR products. A PCR reaction templated by murine *C. trachomatis* (MoPn) DNA was carried out with degenerate oligonucleotides derived from regions 2.1 and 2.2 (primers 1 and 2) as the 5′ primers and degenerate oligonucleotides derived from region 2.3 (primer 3) as the 3′ primers. Either 5′ primer used in conjunction with the 3′ primer gave a single product as visualized by agarose gel electrophoresis (Fig. 2, lanes 2 and 3). The product from a PCR reaction with primers 2 and 3 on chlamydial DNA was identical in size to that generated in a parallel PCR reaction templated by *E. coli* DNA (Fig. 2, lane 1), and the product generated by using primers 1 and 3 on MoPn DNA yielded an amplified fragment of the size that would be predicted to occur if *E. coli* DNA had been used as the template.

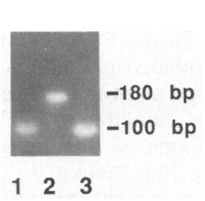

FIG. 2. Agarose gel electrophoresis of the PCR reaction products. One-tenth of the reaction products from a PCR reaction primed with primers 2 and 3 in the presence of *E. coli* DNA (lane 1) or chlamydial DNA (lane 3) or with primers 1 and 3 in the presence of chlamydial DNA (lane 2) were electrophoresed on a 1.5% low-melting-point agarose (Seaplaque) gel, stained with ethidium bromide, and photographed.

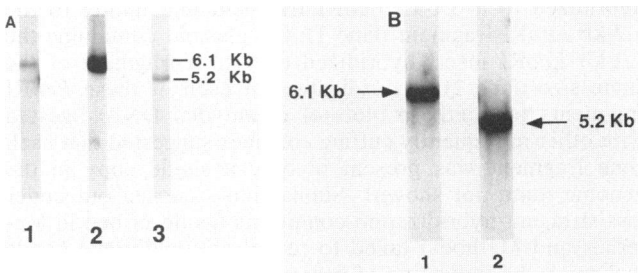

FIG. 3. (A) Southern blot analysis of chlamydial DNA and the cloned putative sigma factor fragments probed with the PCR reaction products. MoPn DNA (1 μg) (lane 1) or 100 ng of the clone containing the 6.1-kb *Eco*RI fragment (lane 2) or the 5.2-kb *Eco*RI fragment (lane 3) was cleaved by *Eco*RI and electrophoresed on a 1% agarose gel (Seakem). The reaction products from a PCR reaction carried out with primers 1 and 3 in the presence of MoPn DNA were 5′-end labeled by T4 polynucleotide kinase in the presence of [γ-^{32}P]ATP and hybridized to this blot. Southern analysis was carried out as described previously (9). (B) Southern blot analysis of chlamydial DNA hybridized to the cloned putative sigma factor fragments. MoPn DNA (1 μg) was cleaved with *Eco*RI, and electrophoresed on a 1% agarose gel, and hybridized to a probe made by nick-translation of the 6.1-kb *Eco*RI fragment (lane 1) or the 5.2-kb *Eco*RI fragment (lane 2).

The PCR product directed by primers 1 and 3 on MoPn DNA was cloned into the vector pGEM7Zf. Four separate isolates were sequenced and found to be identical; the amino acid sequence derived from the DNA sequence showed 93% identity to sigma-70 of *E. coli* (45 of 48 amino acids residues, data not shown) and 88% identity to sigma-43 of *B. subtilis* (42 of 48 residues; data not shown). This finding suggested that the cloned PCR product corresponded to the chlamydial homolog of the major sigma factor from *E. coli* and *B. subtilis*.

PCR probe hybridizes to two distinct chromosomal fragments. The products of a PCR reaction from MoPn DNA primed with primers 1 and 3 was 5′-end labeled with polynucleotide kinase and [γ-^{32}P]ATP and used as a hybridization probe on a Southern blot of chlamydial DNA digested with *Eco*RI. Two bands of hybridization were observed: a 6.1-kilobase (kb) *Eco*RI fragment (which hybridized strongly to this probe) and a 5.2-kb *Eco*RI fragment (which hybridized less strongly to this probe) (Fig. 3A), lane 1). The 5′-end-labeled PCR probe was then hybridized to an *Eco*RI library of chlamydial DNA cloned into the plasmid vector pUC8. Screening of 900 such plasmids yielded three positive clones. Restriction analysis of their inserts by cleavage with *Eco*RI demonstrated the presence of two classes of inserts: those containing the 6.1-kb *Eco*RI fragment and those containing the 5.2-kb *Eco*RI fragment (data not shown). These two cloned *Eco*RI fragments comigrated with the 6.1- and 5.2-kb bands of hybridization seen on the genomic Southern blot (Fig. 3A, lanes 1, 2, and 3); additionally, the extent of hybridization of the PCR reaction mixture to each subcloned *Eco*RI fragment paralleled the extent of hybridization seen on the Southern blot of genomic DNA. As sequence analysis of a cloned fragment derived from the PCR reaction failed to reveal the presence of an *Eco*RI site between primers 1 and 3 (data not shown), this finding suggested that chlamydiae might encode at least two sigma factor genes.

DNA prepared from a representative member of each class was labeled by nick-translation and hybridized to a Southern blot of MoPn chlamydial DNA cleaved with *Eco*RI (Fig. 3B). The plasmid containing the 6.1-kb *Eco*RI insert

hybridized to a 6.1-kb *Eco*RI fragment and faintly to the 5.2-kb *Eco*RI fragment (lane 1); the plasmid containing the 5.2-kb *Eco*RI insert hybridized only to a fragment of the same size (lane 2). Hybridization of each of these *Eco*RI fragments to Southern blots of chlamydial DNA digested with other infrequently cutting enzymes suggested that each gene fragment was present in only a single copy in the genome (data not shown). Similar blots carried out under less-stringent hybridization conditions (as described in Materials and Methods) failed to reveal any additional bands that might be indicative of other less-related sigma factors (data not shown). Low-stringency hybridization of a nick-translated probe made to the 6.1-kb *Eco*RI fragment to a Southern blot of LGV (serovar L2) DNA digested with a panel of infrequently cleaving restriction enzymes suggested that the gene fragment was conserved in this closely related chlamydial species and was present in a single copy (data not shown).

Sequence of the chlamydial homolog of sigma-70. Preliminary DNA sequence analysis of the terminal *Xba*I-*Eco*RI fragment (p297, see Fig. 6B) derived from the 6.1-kb *Eco*RI fragment indicated the presence at its 3′ end of an open reading frame with strong homology to the N-terminal (approximately) 400 amino acids of *E. coli* sigma-70. Since the open reading frame was interrupted by the *Eco*RI site, we recovered an overlapping restriction fragment containing the 3′ coding portion of the gene by screening an *Sph*I-*Xho*I library of MoPn DNA cloned into pGEM7Zf. The relevant *Eco*RI fragment containing the C-terminal portion of the sigma factor gene from this clone was isolated (the 3′-terminal *Eco*RI site derives from the polylinker) and subsequently ligated into the *Eco*RI site of p297 to yield clone A, which reconstitutes the entire coding region of the putative sigma factor gene (see Fig. 6B). Figure 4 shows the DNA and derived amino acid sequence of the open reading frame encoded by clone A; it potentially specified a protein of 571 amino acids. No close match to a ribosome-binding site (30) was identifiable within 20 base pairs (bp) of the presumed N-terminal methionine codon. If, as in *E. coli* and *B. subtilis*, the gene encoding this sigma factor is part of an operon (3, 35), then a weak ribosome-binding site may suffice to function during translational coupling.

Figure 5 compares the derived amino acid sequence of clone A with that of the sigma-70 gene of *E. coli* (*rpoD*). As might be predicted from the previously described comparisons of bacterial sigma factors, clone A contained sequences that were homologous to regions 1, 2, 3, and 4 from *E. coli* sigma-70 (Fig. 4) and *B. subtilis* sigma-43 (data not shown). Region 2 was most highly conserved, while regions 1, 3, and 4 were less well conserved. Specifically, region 1 showed 35% identity to sigma-70; an additional 40% of the residues were conserved. In fact, region 1 of this chlamydial sigma factor was not significantly more diverged from sigma-70 of *E. coli* than it was from sigma-43 of *B. subtilis* (30% identical and 30% additional conservative changes). Region 2 demonstrated 92% identity and 97% identity or conservation with sigma-70. Regions 3 and 4 were more divergent from sigma-70, 46% identity with 41% additional conserved residues and 67% identity with 17% additional conserved residues, respectively. Both the N-terminus and C-terminus of the chlamydial protein had extensions of 15 amino acids each compared with sigma-70 of *E. coli* and sigma-43 of *B. subtilis*. There was, in addition, a 55-amino-acid deletion in the chlamydial sigma factor compared with sigma-70 that occurred somewhere between residues 135 and 220. Interestingly, *B. subtilis* sigma-43 contains a 245-amino-acid deletion in the same region compared with *E. coli* sigma-70. Based on the homology of clone A to the major sigma factors of *E. coli* (sigma-70) and *B. subtilis* (sigma-43), we suspect that this locus encodes the major vegetative sigma factor from murine *C. trachomatis*; we propose to name this gene *sigA* and the protein encoded by it sigma-A.

***sigA* gene directs the synthesis of a 68-kDa protein in *E. coli*.** Clone A, which contains the *sigA* gene downstream from the bacteriophage T7 promoter, was transformed into a strain of *E. coli* containing a compatible plasmid, pGP1-2, that expresses the T7 polymerase gene under control of a lambda promoter and a thermolabile lambda repressor (*c*I857) (33). In this strain, clone A directed the synthesis of an approximately 68-kilodalton (kDa) protein (Fig. 6A, lane 3) which was not seen without heat induction (lane 2) or in a control plasmid lacking the sigma-A gene (pGEM7Zf alone, lane 1). Further proof that this 68-kDa protein is the product of the *sigA* gene was provided by the following experiment. A plasmid containing either a C-terminal deletion (p297; *Xba*I-*Eco*RI fragment of clone A) or an N-terminal deletion (p335; *Eco*RI-*Xho*I fragment of clone A) was introduced into the expression strain and thermoinduced for protein expression. A smaller protein product (approximately 44 kDa) was seen when a plasmid deleted for the C-terminal portion was heat-induced for protein expression (Fig. 6A, lane 4). The construct deleted for the N-terminus showed (as predicted) no 68-kDa product upon heat induction (p335, lane 5). Several small proteins were prominent in this lane. It seems unlikely that they represent a protein fragment encoded by the N-terminal truncation, as they were not reproducibly induced above the background levels of the low-molecular-mass species present when p333 was heat-induced (data not shown).

Preliminary characterization of clone B. Restriction digests of the 5.2-kb *Eco*RI fragment (hereafter referred to as clone B) were blotted and probed with the 5′-end-labeled PCR products generated with primers 1 and 3; the smallest fragment analyzed that hybridized to the PCR probe was the terminal *Xho*I-*Eco*RI fragment (data not shown). Preliminary sequencing (one strand only) was carried out over most of this 1.5-kb fragment; no homologies to known sigma factor sequences or regions homologous to the PCR primers were observed (data not shown).

DISCUSSION

In this article we outline a general approach to cloning eubacterial sigma factors and apply it to the isolation of a sigma factor protein from the MoPn strain of *C. trachomatis*. Specifically, we have taken advantage of the known sequence conservation of portions of bacterial sigma factor proteins to design degenerate oligonucleotides corresponding to these domains. These synthetic DNA sequences have been used as primers in the PCR reaction to amplify DNA sequences from the chlamydial genome; the PCR products were subsequently used as probes to recover genomic fragments from a library of cloned murine *C. trachomatis* DNA. Sequence analysis of one of these clones reveals striking homology to the sigma-70 protein of *E. coli* and the sigma-43 protein of *B. subtilis*, strongly implying that the locus we have identified encodes the major vegetative sigma factor of murine *C. trachomatis*. Definitive proof will require demonstration that the product of this gene is associated with chlamydial RNA polymerase.

Characterization of the chlamydial transcriptional apparatus is of interest because prior studies have suggested that

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TCTAGAGGGATTTGCCCAATGGGAGCGAGATTTATTAACAGAATCTCGAACAGAACGCT

CTGGCAAAATCGTAATTAAAACGAAAGCTTTTGGAGCCACTCCGTTCTACTATTGTGTA

GTAACGTTAGATAAAGGACCTTTGGCAGAACATGTATTAGGGGTTTTATACCCTGCAAA

AGCAAGTTTTTTTACAAATCTTTCCTATATTTAATGATAAATAGGGATGTCGGTGTAGG

AAGTTTTTCTAGGGCCGAATCTGATCAGCTGGTTTTTTATCTAAAAATCCCTAGATTTC

TTGTTTTCCTTAAGATAAACTGTCACTTTCTAGGCCGATTTTTCCTTAGTTTTAATTTG


        1                                   10
       Met Arg Ile His Thr Leu Asp Ser Gln Arg Ala Asp Ala
TTAGC  ATG CGC ATC CAT ACG CTA GAT AGT CAA CGT GCT GAT GCA

                        20
Ala Gln Glu Glu Glu Ile Gln Arg Lys Leu Glu Glu Leu Val Thr
GCC CAA GAA GAA GAA ATC CAA AGA AAG TTA GAA GAG CTA GTC ACT

    30                                  40
Leu Ala Lys Asp Gln Gly Phe Ile Thr Tyr Glu Glu Ile Asn Glu
CTT GCT AAG GAT CAA GGG TTC ATC ACG TAT GAA GAA ATT AAT GAA

                        50
Ile Leu Pro Pro Ser Phe Asp Ser Pro Glu Gln Ile Asp Gln Val
ATT CTT CCC CCT TCT TTC GAT TCG CCA GAA CAG ATA GAT CAA GTT

    60                                  70
Leu Ile Phe Leu Ala Gly Met Asp Val Gln Val Leu Asn Gln Ala
TTA ATT TTT CTG GCG GGG ATG GAC GTT CAA GTC TTA AAC CAA GCA

                        80
Asp Val Glu Arg Gln Lys Glu Arg Lys Lys Glu Ala Lys Glu Leu
GAC GTA GAG CGG CAG AAA GAA AGA AAA AAA GAA GCT AAA GAG CTA

    90                                  100
Glu Gly Leu Ala Lys Arg Ser Glu Gly Thr Pro Asp Asp Pro Val
GAA GGG TTG GCT AAG CGT TCT GAG GGA ACG CCT GAT GAT CCA GTG

                        110
Arg Met Tyr Leu Lys Glu Met Gly Thr Val Pro Leu Leu Thr Arg
CGT ATG TAT CTG AAG GAA ATG GGT ACA GTT CCT CTT CTT ACA AGA

    120                                 130
Glu Glu Glu Val Glu Ile Ser Lys Arg Ile Glu Lys Ala Gln Val
GAA GAG GAG GTG GAG ATT TCT AAA AGG ATA GAA AAA GCT CAG GTA

                        140
Gln Ile Glu Arg Ile Ile Leu Arg Phe Arg Tyr Ser Thr Lys Glu
CAA ATA GAA AGA ATT ATT TTA CGC TTT CGT TAT TCG ACT AAA GAA

    150                                 160
Ala Val Ser Ile Ala Gln Tyr Leu Ile Asn Gly Lys Glu Arg Phe
GCG GTT TCT ATT GCG CAA TAC TTA ATT AAT GGT AAG GAA CGA TTT

                        170
Asp Lys Ile Val Ser     Lys Glu Val Glu Asp Lys Thr His Phe
GAT AAG ATC GTT TCC GNA AAA GAG GTG GAA GAT AAG ACA CAT TTT

    180                                 190
Leu Asn Leu Leu Pro Lys Leu Ile Ser Leu Leu Lys Glu Glu Asp
CTT AAT CTT TTG CCG AAG TTA ATC TCT TTG CTG AAA GAA GAG GAC

                        200
Ser Tyr Leu Glu Glu Arg Leu Leu Ala Leu Lys Asp Pro Ala Leu
TCT TAC TTA GAA GAG CGG CTT TTG GCT TTG AAA GAT CCG GCA CTG

    210                                 220
Ser Lys Gln Asp Gln Ala Lys Leu Asn Asp Glu Leu Glu Lys Cys
TCC AAG CAA GAT CAA GCA AAG TTG AAC GAT GAG CTT GAA AAA TGC

                        230
Arg Ile Arg Thr Gln Ala Tyr Leu Arg Cys Phe His Cys Arg His
CGT ATT CGA ACA CAA GCT TAT CTG AGA TGT TTT CAT TGT CGT CAC

    240                                 250
Asn Val Thr Glu Asp Phe Gly Glu Val Val Phe Lys Ala Tyr Asp
AAT GTC ACG GAA GAT TTT GGA GAA GTT GTT TTT AAA GCT TAT GAC

                        260
Ser Phe Leu Gln Leu Glu Gln Gln Ile Asn Asp Leu Lys Val Arg
TCG TTC TTA CAG TTG GAA CAA CAA ATC AAT GAT TTA AAG GTT CGT

    270                                 280
Ala Glu Arg Asn Lys Phe Ala Ala Ala Lys Leu Ala Ala Ala Arg
GCT GAA AGA AAT AAG TTT GCT GCA GCA AAA CTA GCT GCA GCC CGA

                        290
Arg Lys Leu Tyr Lys Arg Glu Val Ala Ala Gly Arg Thr Leu Glu
CGC AAG CTT TAC AAA CGA GAA GTT GCA GCG GGA CGT ACT CTT GAA

    300                                 310
Glu Phe Lys Lys Asp Val Arg Met Leu Gln Arg Trp Met Asp Lys
GAG TTT AAA AAA GAT GTG CGG ATG TTG CAG CGT TGG ATG GAT AAA

                        320
Ser Gln Glu Ala Lys Gln Glu Met Val Glu Ser Asn Leu Arg Leu
AGC CAG GAA GCA AAG CAA GAG ATG GTG GAA TCC AAC TTA CGT TTG

    330                                 340
Val Ile Ser Ile Ala Lys Lys Tyr Thr Asn Arg Gly Leu Ser Phe
GTG ATC TCT ATC GCG AAA AAA TAT ACC AAC CGA GGG CTG TCT TTC

                        350
Leu Asp Leu Ile Gln Glu Gly Asn Met Gly Leu Met Lys Ala Val
TTG GAT TTG ATT CAA GAA GGA AAT ATG GGC TTA ATG AAA GCT GTT

    360                                 370
Glu Lys Phe Glu Tyr Arg Arg Gly Tyr Lys Phe Ser Thr Tyr Ala
GAA AAA TTT GAG TAT CGC CGG GGT TAT AAA TTT TCA ACT TAT GCC

                        380
Thr Trp Trp Ile Arg Gln Ala Val Thr Arg Ala Ile Ala Asp Gln
ACT TGG TGG ATT CGT CAG GCT GTG ACG CGA GCT ATT GCC GAT CAG

    390                                 400
Ala Arg Thr Ile Arg Ile Pro Val His Met Ile Glu Thr Ile Asn
GCA AGA ACC ATT CGA ATT CCT GTT CAT ATG ATC GAG ACC ATT AAT

                        410
Lys Val Leu Arg Gly Ala Lys Lys Leu Met Met Glu Thr Gly Lys
AAG GTG CTT CGT GGA GCC AAG AAA TTA ATG ATG GAA ACT GGA AAA

    420                                 430
Glu Pro Thr Pro Glu Glu Leu Gly Glu Glu Leu Gly Phe Thr Pro
GAG CCT ACG CCC GAA GAA CTC GGA GAG GAA CTA GGT TTC ACT CCA

                        440
Asp Arg Val Arg Glu Ile Tyr Lys Ile Ala Gln His Pro Ile Ser
GAC CGT GTT CGA GAA ATT TAT AAG ATC GCT CAG CAT CCG ATT TCT

    450                                 460
Leu Gln Ala Glu Val Gly Asp Ser Gly Glu Ser Ser Phe Gly Asp
TTA CAG GCT GAG GTT GGA GAT AGT GGA GAA AGC TCT TTT GGA GAT

                        470
Phe Leu Glu Asp Thr Ala Val Glu Ser Pro Ala Glu Ala Thr Gly
TTC TTG GAA GAT ACA GCT GTT GAA TCT CCG GCA GAG GCA ACA GGC

    480                                 490
Tyr Ser Met Leu Lys Asp Lys Met Lys Lys Val Leu Lys Thr Leu
TAC TCC ATG TTG AAA GAC AAA ATG AAG AAA GTG CTA AAA ACG CTT

                        500
Thr Asp Arg Glu Arg Phe Val Leu Ile His Arg Phe Gly Leu Leu
ACT GAT CGC GAA CGT TTT GTT TTG ATC CAT CGG TTT GGC CTT TTA

    510                                 520
Asp Gly Arg Pro Lys Thr Leu Glu Glu Val Gly Ser Ala Phe Asn
GAT GGC CGT CCC AAA ACT TTG GAA GAG GTA GGC TCC GCG TTC AAC

                        530
Val Thr Arg Glu Arg Ile Arg Gln Ile Glu Ala Lys Ala Leu Arg
GTG ACA CGA GAG CGG ATT CGA CAA ATC GAA GCC AAA GCT TTG CGA

    540                                 550
Lys Met Arg His Pro Ile Arg Ser Lys Gln Leu Arg Ala Phe Leu
AAA ATG CGT CAT CCT ATC CGT TCC AAA CAG CTA CGA GCA TTC TTG

                        560
Asp Leu Leu Glu Glu Glu Lys Thr Gly Ser Gly Lys Ile Lys Ser
GAT TTA TTG GAA GAA GAG AAG ACT GGT TCG GGC AAG ATT AAG AGT

    570 571
Tyr Lys Asn OC
TAT AAG AAT TAA GGATTCCTTTTCATAAGGAGTATGCTTGTATCGGTTAGACGTAAC

GAACTTTCGTGTTTGGGTATCTATAGGGGTCTCAGAGCAGGAACGCTATCACAAGCAACC
```

FIG. 4. Nucleotide and predicted protein sequence of the *sigA* gene. Only the coding strand is shown. The 5′ end of the nucleotide sequence corresponds to the *XbaI* site (see Fig. 6B).

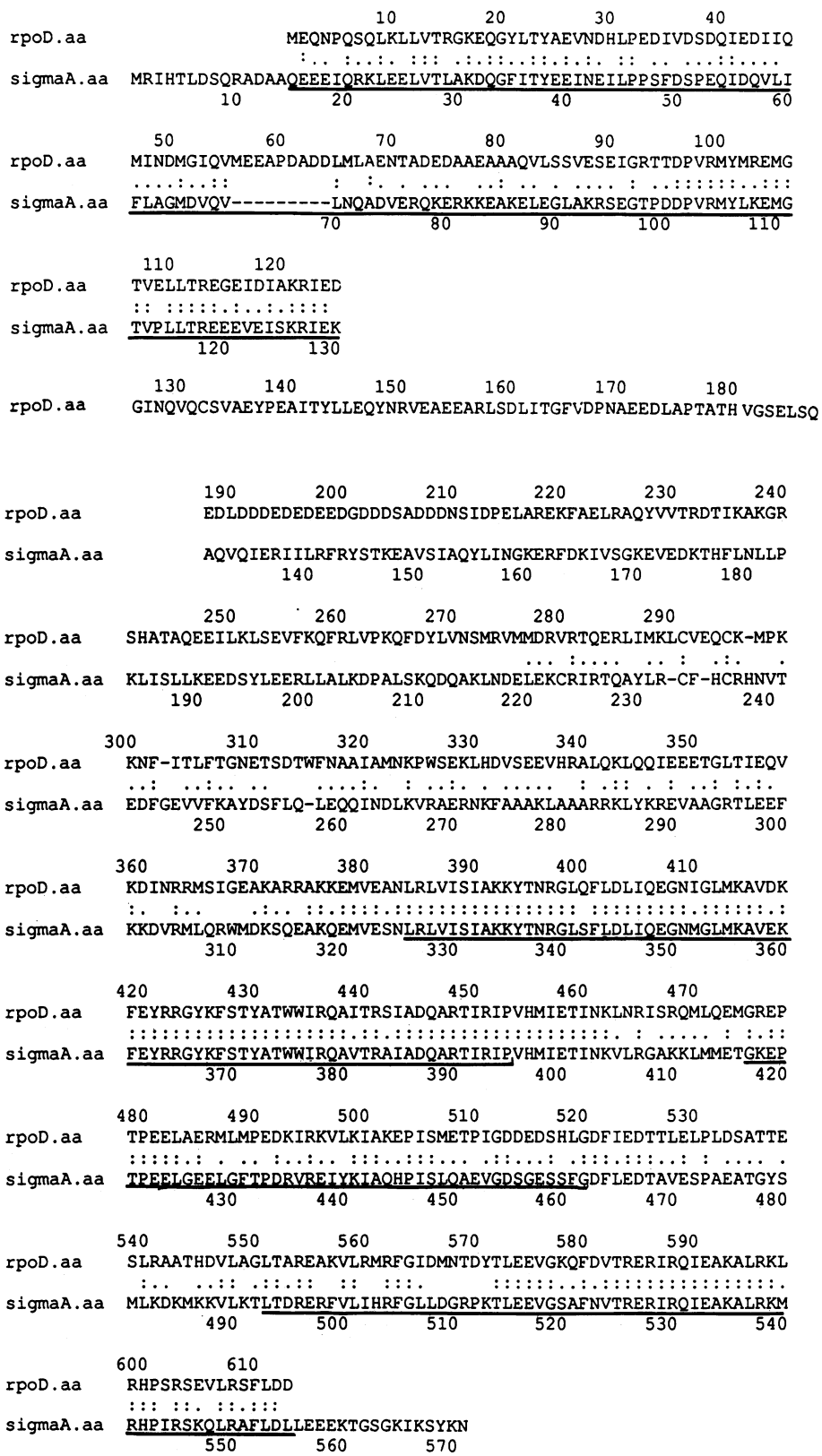

FIG. 5. Comparison of the predicted amino acid (aa) sequence of the murine *C. trachomatis* sigma-A (sigmaA.aa) with that of the *E. coli* sigma-70 (rpoD.aa). The single-letter code for amino acids is used. Dashes represent gaps introduced to maximize sequence similarities. There are two gaps in sigma-A compared with sigma-70, 9 and 55 amino acids in length. The 55-amino-acid deletion is shown to correspond to amino acids 126–190 of sigma-70; however, it could occur anywhere between amino acids 126 and 280 of sigma-70. Double dots indicate amino acid identity; single dots indicate conservative changes. The four underlined regions correspond to the conserved regions 1 (amino acids 1 to 125), 2 (amino acids 384 to 453), 3 (amino acids 475 to 520), and 4 (amino acids 551 to 613) of *E. coli* sigma-70 (15).

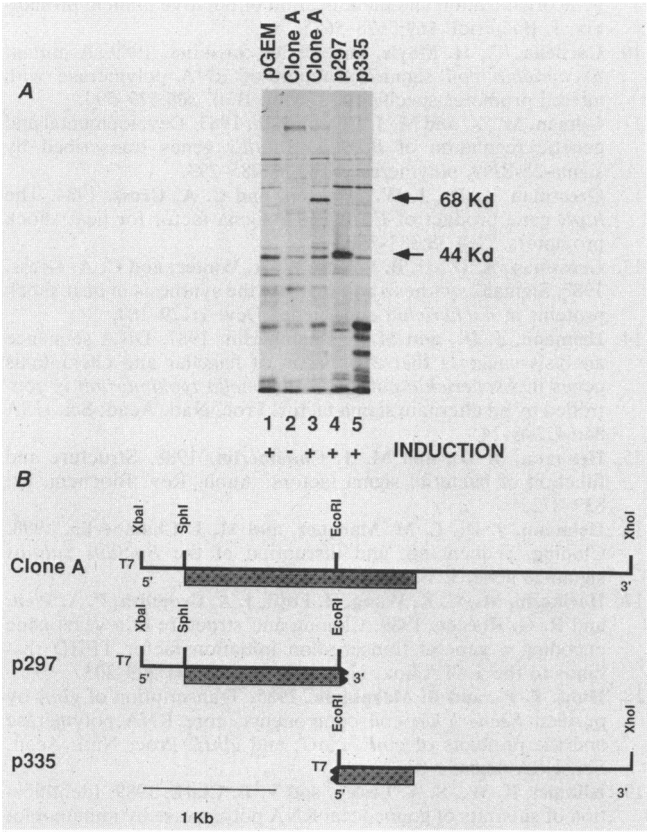

FIG. 6. Expression of the sigma-A protein in *E. coli*. Plasmid-encoded gene products were expressed in a strain of *E. coli* containing pGP1-2 (33) and one of the following plasmids: pGEM7Zf (lane 1), clone A (lanes 2 and 3), p297 (lane 4), or p335 (lane 5), as described in Materials and Methods. The proteins in lanes 1, 3, 4, and 5 were from bacteria that had been thermoinduced for plasmid protein expression from the T7 polymerase promoter in pGEM7Zf. A 1-ml portion of cells was used; after pelleting, the cells were suspended in 100 μl of Laemmli buffer (20), and 10 μl was electrophoresed on a 12% polyacrylamide–SDS gel (1). (A) Autoradiograph of the SDS-polyacrylamide gel. (B) Relevant restriction sites in the clones used for expression of the sigma-A protein in *E. coli*. The shaded region represents the coding region of the *sigA* gene; the hatched ends delimit the extent of the deleted coding region in the truncated constructs. The construction of the clones is described in the text. Kd, Kilodaltons.

chlamydial promoter sequences differ from those previously characterized in other bacteria (9, 27, 32; Engel and Ganem, in press). DNA sequence analysis of the gene encoding the chlamydial sigma factor has shown that protein domains conserved among sigma factors from other eubacteria are conserved in this homolog. It is perhaps surprising that regions 2.4 (−10 binding) and 4 (−35 binding) are conserved compared with the major sigma factors from *E. coli* and *B. subtilis*, because the five putative promoter regions from three chlamydial genes that we have previously characterized have −10 and −35 sequences significantly diverged from those in *E. coli* (9; Engel and Ganem, in press). Genetic analysis has suggested that the arginine residues found at amino acids 584 and 588 of sigma-70 are important in the binding of the *E. coli* holoenzyme to the −35 sequence; analysis of suppressor mutants localized the interaction to the cytidine residue of the sequence TTGACA (10, 15, 31). Surprisingly, these two amino acids are conserved in sigma-A, although the −35 regions of the five putative chlamydial promoters we have analyzed do not always conserve the cytidine residue of TTGACA. It is formally possible that sigma-A is not part of the holoenzyme responsible for transcription from these five promoters. We think this unlikely, since at least two of these three genes (encoding the rRNAs and S1 protein) are transcribed by the sigma-70-containing holoenzyme in *E. coli*. Of note is the observation that the recently cloned TATAA-binding protein from a much more evolutionarily divergent organism, *Saccharomyces cerevisiae*, still exhibits protein homology to region 2.4 (17).

The *sigA* gene directs the synthesis of a 68-kDa protein product when expressed in *E. coli*. Given the 32-amino-acid difference in predicted length between the chlamydial sigma-A protein and *E. coli* sigma-70, this apparent molecular mass is in accordance with our data. The strain of *E. coli* capable of expressing the *sigA* protein product grows well at 30 or 42°C (a temperature at which the *sigA* gene product is induced), suggesting that the expression of the chlamydial sigma-A protein does not disrupt the *E. coli* transcriptional apparatus (J. Engel, unpublished observations). Several explanations for this finding are possible. Of note, the chlamydial sigma factor appears to be stable in this strain of *E. coli* (J. Engel, unpublished observations). Perhaps sigma-A cannot associate with the *E. coli* holoenzyme. Alternatively, sigma-A may exhibit low-affinity binding to *E. coli* core enzyme, leaving the majority of the holoenzyme intact and transcriptionally active. We are currently conducting experiments to determine whether the purified chlamydial sigma-A protein can associate with *E. coli* core enzyme in vivo or in vitro.

Attempts to characterize the chlamydial sigma factor promoter itself and to localize the 5′ end of the sigma-A mRNA by Northern blot analysis, S1 nuclease analysis, and primer extension experiments have been unsuccessful (J. Engel, unpublished observations). The lack of a signal detectable by Northern blotting suggests that the mRNA is either of very low abundance or very unstable. The inability to map the 5′ end of the mRNA may be due to similar reasons. Alternatively, as in *E. coli* and *B. subtilis* (3, 35), this sigma factor may be part of an operon, making detection of the primary transcript difficult. It is likely that an analysis of the temporal expression of this protein will require an approach utilizing antibodies to the sigma factor.

A putative second chlamydial sigma factor gene was also isolated during this screen. DNA sequencing, however, failed to reveal any discernible homology to previously characterized eubacterial sigma factors. This paradox has two possible explanations: either the hybridization of the PCR-amplified sequences to the 5.2-kb *Eco*RI fragment was fortuitous (i.e., the result of nonspecific annealing mediated by G+C-rich sequences), or, less likely, this gene fragment encodes a sigma factor that is highly diverged from the major vegetative sigma factors of Enterobacteriaceae. To prove that this gene fragment could indeed encode a sigma factor, it will be necessary to demonstrate that its protein product is associated with the chlamydial RNA polymerase holoenzyme either by direct purification or by immunoblotting with a specific antibody.

Our failure to recover a host of other sigma factors from *C. trachomatis* may reflect the presence of only a sigma factor in this procaryote. Alternatively, since the degenerate oligonucleotides were designed to detect protein domains primarily conserved between sigma-70 and sigma-43, this result may simply indicate that the other chlamydial sigma factors

are highly diverged from the major vegetative sigma proteins from *E. coli* and *B. subtilis*. Using a synthetic oligonucleotide (derived from region 2) as a probe, Tanaka et al. (34) have recently shown that multiple genes potentially encoding sigma-70 homologs are found in *Micrococcus*, *Pseudomonas*, and *Streptomyces* spp., whereas single genes were detected in *E. coli*, *B. subtilis*, and *Staphylococcus aureus*. This oligonucleotide was selected to specifically detect sigma-70 homologs, as it derives from a region of the sigma-70 protein that is not highly conserved in the alternative sigma factors (15).

The characterization of sigma factors from other eubacteria has depended on direct biochemical purification of bacterial polymerase combined with assaying the holoenzyme by in vitro transcription on specific templates. Such an approach is not feasible in chlamydiae; the poor growth of this organism in culture makes it exceedingly difficult to generate the necessary starting material for such large-scale enzyme purifications. Our approach of using PCR to isolate sigma factor genes from chlamydiae followed by their direct expression in *E. coli* provides an attractive alternative method for the identification and characterization of the components of the chlamydial transcriptional machinery. We have recently applied this PCR-based approach to successfully clone the β and β′ subunits of murine *C. trachomatis* RNA polymerase (J. Engel, J. Pollack, F. Malik, and D. Ganem, manuscript in preparation). Finally, we note that this method should have broad application to the isolation of major sigma factor genes from most, if not all, eubacterial species.

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LITERATURE CITED

1. **Baehr, W., M. J. Devlin, and M. L. Applebury.** 1979. Isolation and characterization of a cGMP phosphodiesterase from bovine rod outer segments. J. Biol. Chem. **254**:11669–11677.
2. **Becker, Y.** 1978. The chlamydia: molecular biology of procaryotic obligate parasites of eucaryotes. Microbiol. Rev. **42**:274–306.
3. **Burton, Z. F., C. A. Gross, K. K. Watanabe, and R. R. Burgess.** 1983. The operon that encodes the sigma subunit of RNA polymerase also encodes ribosomal protein S21 and DNA primase in *E. coli* K12. Cell **32**:335–349.
4. **Buttner, M. J., A. M. Smith, and M. J. Bibb.** 1988. At least three different RNA polymerase holoenzymes direct transcription of the agarase gene (*dagA*) of *Streptomyces coelicolor* A3(2). Cell **52**:599–609.
5. **Chater, K. F., C. J. Bruton, K. A. Plaskitt, M. J. Buttner, C. Mendez, and J. D. Helmann.** 1989. The developmental fate of *S. coelicolor* hyphae depends upon a gene product homologous with the motility sigma factor of *B. subtilis*. Cell **59**:133–143.
6. **Doi, R. H.** 1982. Multiple RNA polymerase holoenzymes exert transcriptional specificity in *Bacillus subtilis*. Arch. Biochem. Biophys. **214**:772–781.
7. **Doi, R. H., S. M. Halling, V. M. Williamson, and K. C. Burtis.** 1980. The transcriptional apparatus of *Bacillus subtilis* RNA polymerase: a model for Gram positive prokaryotes, 117–136. *In* S. Osawa, H. Ozeki, H. Uchida, and T. Yura (ed.), Genetics and evolution of RNA polymerase, tRNA, and ribosomes. University of Tokyo Press, Tokyo.
8. **Doi, R. H., and L. F. Wang.** 1986. Multiple procaryotic ribonucleic acid polymerase sigma factors. Microbiol. Rev. **50**:227–243.
9. **Engel, J. N., and D. Ganem.** 1987. Chlamydial rRNA operons: gene organization and identification of putative tandem promoters. J. Bacteriol. **169**:5678–5685.
10. **Gardella, T., H. Moyle, and M. M. Susskind.** 1989. A mutant *Escherichia coli* sigma70 subunit of RNA polymerase with altered promoter specificity. J. Mol. Biol. **206**:579–590.
11. **Gilman, M. Z., and M. J. Chamberlin.** 1983. Developmental and genetic regulation of *Bacillus subtilis* genes transcribed by sigma-28-RNA polymerase. Cell **35**:285–293.
12. **Grossman, A. D., J. W. Erickson, and C. A. Gross.** 1984. The *htpR* gene product of *E. coli* is a sigma factor for heat shock promoters. Cell **38**:383–390.
13. **Grossman, A. D., D. B. Straus, W. A. Walter, and C. A. Gross.** 1987. Sigma32 synthesis can regulate the synthesis of heat shock proteins in *Escherichia coli*. Genes Dev. **1**:179–184.
14. **Helmann, J. D., and M. J. Chamberlin.** 1987. DNA sequence analysis suggests that expression of flagellar and chemotaxis genes in *Escherichia coli* and *Salmonella typhimurium* is controlled by an alternate sigma factor. Proc. Natl. Acad. Sci. USA **84**:6422–6424.
15. **Helmann, J. D., and M. J. Chamberlin.** 1988. Structure and function of bacterial sigma factors. Annu. Rev. Biochem. **57**:839–872.
16. **Helmann, J. D., L. M. Marquez, and M. J. Chamberlin.** 1988. Cloning, sequencing, and disruption of the *Bacillus subtilis* sigma-28 gene. J. Bacteriol. **170**:1568–1574.
17. **Horikoshi, M., C. K. Wang, H. Fujii, J. A. Cromlish, P. A. Weil, and R. G. Roeder.** 1989. Cloning and structure of a yeast gene encoding a general transcription initiation factor TFIID that binds to the TATA box. Nature (London) **341**:299–303.
18. **Hunt, T. P., and B. Magasanik.** 1985. Transcription of *glnA* by purified *Escherichia coli* components: core RNA polymerase and the products of *glnF*, *glnG*, and *glnH*. Proc. Natl. Acad. Sci. USA **82**:8453–8457.
19. **Klimpel, K. W., S. A. Lesley, and V. L. Clark.** 1989. Identification of subunits of gonococcal RNA polymerase by immunoblot analysis: evidence for multiple sigma factors. J. Bacteriol. **171**:3713–3718.
20. **Laemmli, U. K., and M. Favre.** 1973. Maturation of the head of bacteriophage T4. I. DNA packaging events. J. Mol. Biol. **80**:575–599.
21. **Losick, R.** 1982. Sporulation genes and their regulation, p. 179–199. *In* D. Dubnau (ed.), The molecular biology of the bacilli. Academic Press, Inc., New York.
22. **Losick, R., and J. Pero.** 1981. Cascades of sigma factors. Cell **25**:582–584.
23. **Losick, R., P. Youngman, and P. J. Piggot.** 1986. Genetics of endospore formation in *Bacillus subtilis*. Annu. Rev. Genet. **20**:625–629.
24. **Maniatis, T., E. F. Fritsch, and J. Sambrook.** 1982. Molecular cloning: a laboratory manual. Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y.
25. **Merrick, M. J., and J. R. Gibbons.** 1985. The nucleotide sequence of the nitrogen-regulation gene *ntrA* of *Klebsiella pneumoniae* and comparison with conserved features in bacterial RNA polymerase sigma factors. Nucleic Acids Res. **13**:7607–7620.
26. **Sanger, K., S. Nicklen, and A. R. Coulson.** 1977. DNA sequencing with chain-terminating inhibitors. Proc. Natl. Acad. Sci. USA **74**:5463–5467.
27. **Sardinia, L. M., J. N. Engel, and D. Ganem.** 1989. Chlamydial gene encoding a 70-kilodalton antigen in *Escherichia coli*: analysis of expression signals and identification of the gene product. J. Bacteriol. **171**:335–341.
28. **Schachter, J.** 1988. The intracellular life cycle of chlamydia. Curr. Top. Microbiol. Immunol. **138**:109–139.
29. **Schachter, J., and H. D. Caldwell.** 1980. Chlamydiae. Annu. Rev. Microbiol. **34**:285–309.
30. **Shine, J., and L. Dalgarno.** 1974. The 3′ terminal sequence of *Escherichia coli* 16S ribosomal RNA: complementarity to nonsense triplets and ribosome binding sites. Proc. Natl. Acad. Sci. USA **71**:1342–1346.
31. **Siegele, D. A., J. C. Hu, W. A. Walter, and C. A. Gross.** 1989. Altered promoter recognition by mutant forms of the sigma70

subunit of *Escherichia coli* RNA polymerase. J. Mol. Biol. **206**:591–603.

32. **Stephens, R. A., E. A. Wagar, and U. Edman.** 1988. Developmental regulation of tandem promoters for the major outer membrane protein of *Chlamydia trachomatis*. J. Bacteriol. **170**:744–750.
33. **Tabor, S., and C. C. Richardson.** 1985. A bacteriophage T7 RNA polymerase/promoter system for controlled exclusive expression of specific genes. Proc. Natl. Acad. Sci. USA **82**:1074–1078.
34. **Tanaka, K., T. Shiina, and H. Takahashi.** 1988. Multiple principal sigma factor homologs in eubacteria: identification of the "*rpoD* box." Science **242**:1040–1042.
35. **Wang, L.-F., and R. H. Doi.** 1986. Nucleotide sequence and organization of *Bacillus subtilis* RNA polymerase major sigma (sigma 43) operon. Nucleic Acids Res. **14**:4293–4307.
36. **Westpheling, J., and M. Brawner.** 1989. Two transcribing activities are involved in expression of the *Streptomyces* galactose operon. J. Bacteriol. **171**:1355–1361.
37. **Westpheling, J., M. Ranes, and R. Losick.** 1985. RNA polymerase heterogeneity in *Streptomyces coelicolor*. Nature (London) **313**:22–27.