Characterization of the Primary \( \sigma \) Factor of Staphylococcus aureus*

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RNA polymerase (RNAP) isolated from Staphylococcus aureus is deficient in \( \sigma \) factor and is poorly active in transcription assays. Based on amino acid sequence homology of the Bacillus subtilis vegetative \( \sigma \) factor \( \sigma^{A} \) and the predicted product of the chromosomally located \( \text{plaC} \) gene of \( S. \) aureus, it was hypothesized that \( \text{plaC} \) could encode the vegetative \( \sigma \) factor. We cloned \( \text{plaC} \) under a T7 promoter and overexpressed it in Escherichia coli strain BL21(DE3)pLysE. The overproduced protein, present in inclusion bodies, was solubilized with guanidine hydrochloride, renatured, and purified by DEAE-Sephadex and Sephadex G-75 chromatography. The purified protein, designated \( \sigma^{SA} \), cross-reacted with the \( B. \) subtilis anti-\( \sigma^{A} \) antibody. \( E. \) coli core RNAP, reconstituted with \( \sigma^{SA} \), initiated promoter-specific transcription from the \( S. \) aureus promoters hla, hsa, and sec and from the \( E. \) coli promoters rpoH P1, rpoH P4, and ColE1 RNA-1, which are recognized by the E. coli \( \sigma^{70} \), \( \sigma^{SA} \), when added to the purified RNAP from \( S. \) aureus, stimulated transcriptional activity of the RNAP up to 72-fold. As determined by primer extension studies, the 5'-ends of the \( \sigma^{SA} \)-initiated mRNAs synthesized in vitro from the agr P2 and sea promoters are in general agreement with the 5'-ends of the cellular RNAs. Disruption of the \( \text{plaC} \) gene on the \( S. \) aureus chromosome was lethal. We conclude that \( \text{plaC} \) encodes the primary \( \sigma \) factor in \( S. \) aureus.

Staphylococcus aureus is a common human and animal pathogen (1, 2). The pathogenesis of \( S. \) aureus is primarily the result of secretion of a large number of extracellular and cell wall-associated proteins that facilitate the colonization, multiplication, and spread of the bacterium (3, 4). Three global elements, the accessory gene regulator (agr) (5–8), the exoprotein regulator (xpr) (9, 10), and the Staphylococcal accessory regulator (sar) (11–13), have recently been implicated in the temporal and coordinate expression of these exoproteins. Genetic studies have revealed that the regulation of exoprotein gene expression by these global elements is at the level of transcription. To gain further insights into the complicated mechanism of transcriptional regulation, it is necessary to study transcription in vitro. In all bacterial systems studied, the RNA polymerase (RNAP)\(^{5} \) core enzyme is composed of four subunits: two identical \( \alpha \) subunits, \( \beta \), and \( \beta' \). The association of \( \sigma \) to the core allows the RNAP holoenzyme to recognize promoter elements and initiate transcription from specific sites (14, 15). The availability of RNA polymerase and different \( \sigma \) factors from Escherichia coli (16–18) and Bacillus subtilis (19–23) have led to a very advanced level of understanding of gene regulation in these organisms. Biochemical studies on transcriptional regulation in \( S. \) aureus have not progressed to a comparable degree due to the lack of a defined in vitro transcription system. Only recently, RNAP from \( S. \) aureus has been purified and used in in vitro transcription studies (24, 25). The RNAP purified from exponentially growing \( S. \) aureus cells contains only small amounts of \( \sigma \) subunit and is poorly active in transcription reactions. An overexpression system for obtaining large quantities of the \( \sigma \) factor from \( S. \) aureus will be very helpful in elucidating the detailed mechanism of the regulation of gene expression in this organism.

Recently, a subunit of the purified \( S. \) aureus RNAP was identified as the putative \( \sigma \) factor based on its ability to cross-react with the \( B. \) subtilis anti-\( \sigma^{A} \) antibody (24) and the \( E. \) coli anti-\( \sigma^{70} \) antibody (25). The purified protein conferred on the \( E. \) coli core RNAP the ability to initiate authentic transcription from the sea promoter of \( S. \) aureus (25). The protein encoded by the \( S. \) aureus \( \text{plaC} \) gene was predicted to encode the primary \( \sigma \) factor in \( S. \) aureus based on the amino acid sequence identity of the \( \text{plaC} \) gene product with that of the \( B. \) subtilis vegetative \( \sigma \) factor, \( \sigma^{A} \) (26). In this paper, we report the overproduction and purification of the \( \text{plaC} \)-encoded protein and demonstrate that the overproduced protein is the vegetative \( \sigma \) factor.

MATERIALS AND METHODS

Strains and Plasmids—A list of plasmids used is given in Table I. The \( E. \) coli strain BL21(DE3)pLysE and the vector pET-24a(+) used for cloning and overproduction of the \( \text{plaC} \) gene were from Novagen, Inc.

Reagents—Lysostaphin was obtained from Bristol Myers Squibb Company. Restriction enzymes were from Life Technologies, Inc. and New England Biolabs. \( E. \) coli \( \sigma^{70} \) holoenzyme was purchased from Pharmacia Biotech Inc., and the \( E. \) coli RNA polymerase core enzyme was from Epicenter Technologies. Radioactive nucleotides were from either ICN Biomedicals or from Amersham Life Sciences.

Purification of \( S. \) aureus RNA Polymerase—The RNAP enzyme was purified from the \( S. \) aureus strain RN4220 by a modification of published protocols (24, 25). The cells were grown in nutrient broth containing 2% casein enzymatic hydrolysate (Sigma) and 1% yeast extract (Difco). Cells were harvested at \( A_{600} \) of 1.0 by centrifugation (8,000 rpm in a Sorvall GSA rotor) and washed, first with buffer A (10 mM Tris-HCl (pH 7.9), 10 mM MgCl\(_2\), 1.0 M NaCl, 5 mM EDTA (pH 8.0), 0.2 mM DTT, and 5% glycerol) and then with the grinding buffer (29). Cells (25 g, wet weight) were resuspended in 50 ml of grinding buffer containing 5 mg of lysostaphin and incubated at room temperature for 30 min. The cells were then lysed by passing through a French pressure cell at 14,000–16,000 p.s.i. The cell debris was removed by centrifugation (14,000 rpm SS-34 rotor), and polyethyleneimine (Sigma) was added to 0.6% (v/v) to precipitate nucleic acids and bound proteins. Proteins weakly bound to nucleic acids were removed by washing with TGED (10 mM Tris-HCl (pH 7.9), 5% glycerol, 0.1 M EDTA, and 0.2 mM DTT) plus 0.45 M NaCl. RNAP was eluted from nucleic acids with TGED, 1.0 M NaCl. After

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The abbreviations used are: RNAP, RNA polymerase; DTT, dithiothreitol; \( \sigma^{SA} \), primary \( \sigma \) factor from \( S. \) aureus.

\( \sigma^{SA} \)}
ammonium sulfate precipitation (35 g/100 ml) the proteins were resuspended in TGED, 0.1 M NaCl and applied to a heparin-Sepharose column (Sigma) preequilibrated with TGED, 0.1 M NaCl. The column was washed with TGED, 0.2 M NaCl, and the bound proteins were eluted off the column by applying a linear gradient from 0.2 to 1 M NaCl. The fractions containing RNAP subunits were pooled, concentrated by using Centricon-10 concentrators, and loaded onto a Sephacyr-300 column (Pharmacia Biotech Inc.) preequilibrated with TGED, 0.1 M NaCl, and passed through a Q-Sepharose Fast Flow anion exchange column (Sigma). The column was washed with TGED, 0.2 M NaCl, and the bound proteins were eluted by applying a linear gradient from 0.2 to 1 M NaCl to 1,200 ml with cold TGED buffer. The protein suspension was further diluted to 2,400 ml with cold TGED buffer. DEAE-Sephacel centrifuged at 7,500 rpm in a GSA rotor at 4°C. The supernatant was added ammonium sulfate (42 g/100 ml). The proteins were eluted from the T7 promoter and were stored in small aliquots at –20°C. The amount of protein was quantitated by a BioRad protein determination kit using bovine serum albumin as standard. Protein samples were electrophoresed on 12% SDS-polyacrylamide gels and visualized by staining with Coomassie Blue R-250.

Purification of the S. aureus $\alpha$ Factor from SDS-Polyacrylamide Gels—The $\alpha$ SA protein was eluted from a 12% SDS-polyacrylamide gel as described by Hager and Burgess (31) except that we electroeluted the protein instead of eluting it by diffusion. The subsequent steps for SDS removal and renaturation of the $\alpha$ SA protein were the same as described (31).

Immunoblot Analysis—The proteins separated on SDS-polyacrylamide gels were transferred to a nitrocellulose membrane (Bio-Rad). The ECL Western blotting analysis system (Amersham Corp.) was used for detection of proteins on the membrane. The primary antibody (B. subtilis anti-$\alpha$ polyclonal antibody) was used at a 1:3,000 dilution.

In Vitro Transcription Assay—The purified RNAP enzyme from S. aureus or the E. coli core enzyme with or without $\alpha$ SA was incubated in a microcentrifuge tube for 20 min on ice. To this mixture, 40 ng of plasmid DNA was added and incubated at 35°C for 10 min. A mixture containing 40 ng Tris acetate (pH 7.9), 100 mM NaCl, 5 mM MgCl2, 2 mM DTT, 100 $\mu$g/ml bovine serum albumin, 0.25 mM each of ATP, CTP, and GTP, 0.015 mM UTP, 10 mM of (dNTP)/UTP, 50 $\mu$g/ml heparin, and 0.5 units of Prime RNase Inhibitor (5 Prime, Inc., Boulder, CO) was used in each reaction and incubated at 37°C for 10 min. The reactions were terminated by the addition of 100 $\mu$l of a stop solution (0.4 M ammonium acetate, 20 mM EDTA, 0.3% (w/v) SDS, and 4 $\mu$g of mRNA) and precipitated with 300 $\mu$l of 100% ethanol for 1 h at –20°C. After centrifugation, the pellet was washed with 70% ethanol, resuspended in sequencing gel loading buffer containing 98% deionized formamide, 0.2 M EDTA, 0.025 M sodium citrate, and 0.1% bromphenol blue. The samples were incubated in boiling water for 5 min and electrophoresed in a 6% polyacrylamide gel containing 7 M urea.

Primer Extension Reactions—RNA used for mapping the 5’-end was prepared as described above, but the reactants were scaled up to 10-fold. Heparin was excluded from the reaction mixture. The concentration of each of the nucleosidetriphosphates used was 0.5 mM, and none was radiolabeled. The transcription reactions were terminated by adding 5 units of RNase-free DNase I (Boehringer Mannheim) followed by incubation at 35°C for 10 min. The samples were heated to 90°C for 5 min in inactivate DNase I and extracted twice with phenol-chloroform and then with chloroform. RNA was ethanol-precipitated, washed with 70% ethanol, and resuspended in nuclease-free water.

For primer extension reactions, synthetic dig nucleotide primers (25) were end-labeled with [γ-32P]ATP using T4 polynucleotide kinase (Promega Corp.). Reactions were carried out at 42°C for 45 min with 200 units of Moloney murine leukemia virus reverse transcriptase (Promega) following the manufacturer’s protocol. DNA sequencing was performed using the same primer except that none was radiolabeled. The primer extension products were run on a 7.5% polyacrylamide-urea sequencing gel in parallel with sequencing reactions.

Amino Acid Sequencing—The NH2-terminal amino acid sequencing of the overproduced protein was performed by the method of Matsudaira (32). The proteins were separated on a 12% SDS-polyacrylamide gel and blotted to a Westran polyvinylidene difluoride membrane (Schleicher & Schuell). The membrane was briefly stained with Coomassie Blue and destained. The band corresponding to the overproduced protein was excised, and the NH2 terminal of the protein was sequenced at the Genetic Research Facility of the University of Illinois at Urbana-Champaign using a 477A NH2-terminal protein sequencer (Applied Biosystems).

RESULTS

Overexpression and Purification of the $\alpha$ Factor Encoded by plAC—The predicted protein product of the S. aureus chromosomal gene plAC has 78.8% amino acid sequence identity with the B. subtilis SigA, the vegetative $\alpha$ factor (26). To characterize whether the plAC gene product functions as a $\alpha$ factor in S. aureus, we attempted to clone plAC under the control of the T7 promoter, as was done in the case of the B. subtilis sigA gene (30). Unlike the cloning of B. subtilis sigA, when the S. aureus plAC DNA was ligated with the appropriate vector for expression from the T7 promoter and was transformed into the E. coli strain BL21(DE3), we were unable to obtain any stable transformant. We have successfully used the E. coli strain BL21(DE3)pLysE (BL21(DE3)pLysS was not suitable for over-
expression of plaC (Fig. 1). Substantial levels of T7 lysozyme accumulate in this strain. Any T7 RNAP produced from the "leaky" lacUV5 promoter is readily inactivated by the lysozyme in the uninduced state, thus preventing the expression of the cloned gene product that might be toxic for E. coli growth. In our case apparently, the plaC gene product is toxic for E. coli growth. The presence of resident lysozyme also makes it much easier to lyse the cells for the preparation of the cell extracts. The overproduced plaC gene product obtained from isopropyl-β-D-thiogalactopyranoside-induced cell lysate was mostly present in inclusion bodies and migrated on a polyacrylamide gel at a mobility corresponding to 55 kDa (Fig. 1, lane 2). The proteins present in the inclusion bodies were solubilized with 6 M guanidine hydrochloride and chromatographed successively on DEAE Sephacel and G-75 Sephadex columns (Fig. 1, lanes 3 and 4). At this stage of purification, the protein preparation was about 85% pure (Fig. 1, lane 4). Approximately 3 mg of protein was obtained from 2 liters of cell culture. However, some contaminants were present after chromatography on Sephadex G-75 gel filtration. The overproduced protein was purified to near homogeneity from a preparative SDS-polyacrylamide gel (Fig. 1, lane 5). The experimentally determined N-terminal sequence of the overproduced protein (XSDNTVKIKKKQ) matched with the predicted amino acid sequence of the plaC gene product, except that it lacked the first amino acid, methionine.

Immunoblot Analyses—Western blot analyses revealed that the overproduced protein cross-reacted with a polyclonal antibody raised against B. subtilis σA (Fig. 2, lane 2). The cross-reactive band co-migrated with σA of B. subtilis (Fig. 2, compare lanes 2 and 3). We previously identified a 55-kDa subunit of the purified S. aureus RNAP as a candidate for the vegetative σ factor based on its cross-reactivity with anti-σA antibody (24). Western blot analysis showed that the overproduced protein and the 55-kDa subunit of the purified S. aureus RNAP had the same antigenicity, and they co-migrated (Fig. 2, lanes 1 and 2). It is also apparent from the Western blot analyses that the purified S. aureus RNAP contains negligible amounts of the 55-kDa subunit (Fig. 2, lanes 2 and 3). Although the amount of purified S. aureus RNAP used in lane 2 is 25-fold higher than the amount of B. subtilis RNAP used in lane 3, the intensity of the 55-kDa band in lane 2 is almost 5-fold less than the co-migrating σA protein band in lane 3.

Promoter-Specific Transcription by the Purified plaC Gene Product—To investigate the ability of the plaC gene product to act as a σ factor, we reconstituted RNAP holozyme by adding the purified protein to the core RNAP from E. coli (henceforth...
referred to as heterologous RNAP) and assayed for transcriptional activity from different promoters present on supercoiled plasmids. The results of in vitro transcription are shown in Fig. 3. Three separate experiments gave similar results. Note that all of the transcription reactions were performed in the presence of heparin, unless indicated otherwise, to prevent reinitiation of transcription. The purified plaC gene product confers on the core RNAP the ability to initiate specific transcription from several *S. aureus* promoters. The transcripts produced from the three *S. aureus* promoters *sea*, *sec*, and *hla* were of the same size as previously reported using the *s* factor purified from exponentially growing *S. aureus* cells (25). Neither the core RNAP alone nor the purified protein was able to initiate any specific transcript from any of the promoters tested. The sizes of the transcripts synthesized by the *E. coli* Eσ70 from the *sea* and *hla* promoters are identical to those produced by the heterologous RNAP (Fig. 3, compare lane 8 with lane 9, and lane 14 with lane 15). Note that unlike the heterologous RNAP, the *E. coli* Eσ70 failed to produce a specific transcript from the *sec* promoter (Fig. 3, compare lanes 11 and 12). Based on the above results, the plaC-encoded gene product is designated as *s*SA (*S. aureus*-derived *s* factor).

We also tested the ability of the purified *s*SA protein to initiate specific transcription from several *E. coli* promoters.
The plasmid pUC19spf', used to clone the different promoters and their flanking DNA elements, contains the $\sigma^{70}$-dependent ColE1 RNA-1 promoter (27). Both the E. coli $E\sigma^{70}$ and the heterologous RNAP produced the expected 102- and 108-nucleotide transcripts from the RNA-1 promoter (Fig. 3) (27). Transcription was also detected from the $\sigma^{70}$-dependent rpoH P1 (428-nucleotide) and rpoH P4 (290-nucleotide) promoters (33) present on the plasmid pJET41 (Fig. 3, lanes 2 and 3). Note that the E. coli $E\sigma^{70}$ RNAP transcribed from the rpoH P1 promoter more efficiently than the heterologous RNAP, but the transcription from the rpoH P4 promoter was equally efficient for the $E\sigma^{70}$ RNAP or the heterologous RNAP. Neither the heterologous RNAP nor the E. coli $E\sigma^{70}$ produced any transcript from the E. coli $\sigma^{E}$-dependent rpoH P3 promoter (295 nucleotides) (27, 33), present on the pJET41 plasmid (Fig. 3, lanes 2 and 3). We also did not detect any transcript from the E. coli $\sigma^{32}$-dependent dnaK P1 promoter (28) (data not shown).

Concentration-dependent Stimulation of Transcription Activity of the S. aureus RNA Polymerase by $\sigma^{3A}$.—While the specific initiation of transcription from several S. aureus promoters by the overproduced protein establishes that it is a $\sigma$ factor, the RNAP core enzyme used in the above transcriptional assays was from E. coli, a Gram-negative bacterium. For biologically relevant studies, it is desirable to evaluate the role of the $\sigma$ factor in the homologous RNAP holoenzyme (all of the RNAP subunits derived from S. aureus). Consistent with a report from another laboratory (25), the RNAP that we prepared from S. aureus contains trace amounts of the $\sigma$ subunit and is poorly active in vitro transcription reactions. We chose two S. aureus promoters, sea and agr P2, to study the effect of $\sigma^{3A}$ on the purified RNAP from S. aureus. As shown in Fig. 4, a high concentration of the purified RNAP was required for detection of transcripts from the sea promoter. However, the addition of $\sigma^{3A}$ stimulated transcription from the sea promoter by about 72-fold (Fig. 4, compare lanes 5 and 10) and that from the agr P2 promoter by about 30-fold (data not shown).

Having the above results, we titrated the concentration of $\sigma^{3A}$ required to stimulate optimal transcriptional activity of our purified RNAP preparation from S. aureus. The concentrations of purified RNAP used for these assays did not result in any detectable transcriptional activity from both the sea and agr P2 promoters in the absence of any added $\sigma^{3A}$ (Figs. 5A, lane 1, and data not shown), and the trace amounts of $\sigma^{3A}$ present in the purified RNAP are ignored for the analyses of data presented below. There is a linear relationship between the increase in transcriptional activity and the increasing concentrations of $\sigma^{3A}$ added. The peak transcriptional activity was reached at a $\sigma^{3A}$:core molar ratio of approximately 7 ± 1, for both the sea and agr P2 promoters (Figs. 5B and data not shown).

Analyses of the Transcriptional Start Sites of the sea and agr P2 Promoters.—The 5'-ends of the $\sigma^{3A}$-initiated mRNAs synthesized in vitro from the sea and agr P2 promoters were mapped to compare in vitro and in vivo transcription start sites. There are three putative promoter elements, P2a, P2b, and P2c, within the cloned agr P2 promoter region (Fig. 6A). Five major primer extension products were obtained from the mRNA synthesized in vitro using the plasmid DNA containing the agr P2 region as the DNA template. Three of these products might have resulted from the mRNA synthesized from the P2a promoter. We do not know whether this is due to “stuttering” of RNAP in vivo or a similar effect of the reverse transcriptase in

![Fig. 6. Primer extension analyses. The primer extension assays of in vitro synthesized RNA and DNA sequencing analysis were done as described under “Materials and Methods.” A, agr P2 promoter. G, A, T, and C represent the nucleotide sequencing ladder. The primer extension products are marked as P2a, P2b, and P2c. The upstream sequence of the coding strand of the agr P2 gene (5, 34) is shown on the left. The nucleotide corresponding to each of the primer extension products is marked by an asterisk (+). Three bands in the P2a region correspond to the different transcription start sites indicated by + on the left. The potential -10 and -35 sequences for each of the transcription start sites are underlined. B, mapping of the 5'-ends of the sea transcript. The sequencing ladder (A, T, and C) is shown. The primer extension product, and the -10 and -35 sequences (35) are indicated as above. The nucleotide corresponding to the 5'-end of sea mRNA determined from this analysis is indicated as + 1. The asterisk denotes the transcription start site of the in vivo synthesized m-RNA, previously reported (35).](http://www.jbc.org/)

The primer extension analyses showed the nucleotide sequences corresponding to each of the transcription start sites indicated by + on the left. The potential -10 and -35 sequences for each of the transcription start sites are underlined. B, mapping of the 5'-ends of the sea transcript. The sequencing ladder (A, T, and C) is shown. The primer extension product, and the -10 and -35 sequences (35) are indicated as above. The nucleotide corresponding to the 5'-end of sea mRNA determined from this analysis is indicated as + 1. The asterisk denotes the transcription start site of the in vivo synthesized m-RNA, previously reported (35).
vitro. Reported results of 5'-end mapping of in vivo synthesized RNA are somewhat ambiguous. S1 nuclease data suggested one transcript from the agr P2 promoter corresponding to the toposm band indicated in the P2a region (Fig. 6A) (5, 34). Previously reported primer extension data suggested one transcript corresponding to the smallest RNA product in the P2a region (Fig. 6A) and another transcript corresponding to that indicated by P2c (25). The primer extension product P2b (Fig. 6A), obtained using in vitro synthesized RNA as the template, is in agreement with that reported previously (25). Since the P2b product is not detected in vivo, it might be an artifact of the in vitro experiment, or transcription from this promoter is normally down-regulated or repressed in vivo.

Primer extension with in vitro produced mRNA from the sea promoter revealed that the transcription start site corresponded to a guanosine nucleotide (Fig. 6B), which is one nucleotide upstream of the start site observed with the in vivo synthesized mRNA (35). The above results confirm that the $\sigma^{SA}$ factor directs the S. aureus RNAP to initiate specific transcription from the cognate promoters.

**DISCUSSION**

We have cloned the gene encoding the putative vegetative $\sigma$ factor of S. aureus and have demonstrated that the cloned gene product can functionally replace the $\sigma$ factor isolated from exponentially growing S. aureus cells. This is the first $\sigma$ factor from S. aureus that has been overexpressed and successfully used for in vitro transcription studies. Note that the S. aureus RNAP isolated from exponentially growing cells is virtually a core enzyme, nearly devoid of the $\sigma$ factor and poorly active in transcription assays (Figs. 2 and 4A). The purification of the $\sigma$ factor from E. coli cells, with the cloned gene, is very efficient and far less labor intensive as compared with that from S. aureus cells (Fig. 1) (25).

We confirmed that the purified protein is encoded by plaC by matching its amino-terminal 10-amino acid sequence with that of the predicted protein. The predicted plaC gene product has a molecular weight of 42,177, which is close to the calculated molecular weight of 42,957 of the B. subtilis sigA gene product. Note that $\sigma$ factors migrate abnormally on SDS-polyacrylamide gels because of highly positive and negative charge clusters (36, 37). Both the plaC gene product $\sigma^{SA}$ and the B. subtilis $\sigma^{A}$ migrate in gels as 55-kDa proteins (Fig. 2) (30). The common antigenic nature of the purified protein and the $\sigma^{A}$ subunit of the purified B. subtilis RNAP and their co-migration in a polyacrylamide gel support the authenticity of the purified plaC gene product.

Several different criteria establish that the overproduced plaC gene product acts as a $\sigma$ factor, designated as $\sigma^{SA}$. The overproduced $\sigma^{SA}$ enables the core RNAP from E. coli to initiate promoter-specific transcription (Fig. 3). Note that although the transcription specificities of E. $\sigma^{70}$ and E. $\sigma^{SA}$ RNAPs were similar for several S. aureus and E. coli promoters, there were both qualitative and quantitative differences in activities between the two forms of the RNAPs. The quantitative difference is evident from the 428-nucleotide $\text{rpoD}$ P1 promoter transcript (Fig. 3). The qualitative difference is obvious from the fact that E. $\sigma^{70}$ did not transcribe from the sec promoter and that E. $\sigma^{SA}$ gave a transcript corresponding to the in vivo synthesized mRNA (Fig. 3). The activity of the purified S. aureus RNAP enzyme was enhanced by almost 70-fold (sea promoter, Fig. 4) or 30-fold (agr P2 promoter, data not shown) by the addition of $\sigma^{SA}$, and the activity increased in a concentration-dependent fashion with the addition of $\sigma^{SA}$ (Fig. 5B). The primer extension results with the RNA synthesized in vitro, using S. aureus RNAP supplemented with $\sigma^{SA}$, are in general agreement with previously reported data on 5'-end mapping of in vivo synthesized RNA (see "Analyses of the Transcriptional Start Sites of the sea and agr P2 Promoters" under "Results"). All of these results taken together convincingly demonstrate that the $\sigma^{SA}$ protein acts as a $\sigma$ factor in S. aureus.

What is known about the functional role of plaC in cells? A point mutation in plaC, plaC1 in the S. aureus chromosome, resulted in an increase in the copy number of the plasmid pT181 by partially derepressing the synthesis of its counter transcript RNA (38). Note that the plaC1 mutation results in a change of proline to serine (209th amino acid residue from the NH$_2$ terminus) in the conserved — 10 recognition element of $\sigma^{70}$ class of proteins (26). The plaC1 mutation also resulted in the decrease in agr P2 promoter activity. However, several other promoter activities tested under plaC1 background were unaffected. The plaC1 mutation did not affect the growth of cells (26). Thus, the plaC1 mutation most likely impairs transcription from a limited number of promoters that direct the expression of genes, whose products are not essential for cell growth.

A GenBankTM data base search using the BLASTP program (39) revealed that $\sigma^{SA}$ has significant homology with putative primary $\sigma$ factors of several Gram-positive organisms (Table II). In Gram-negative bacteria, the gene encoding the primary $\sigma$ factor is a part of a three-gene cluster in an operon consisting of rpsU (encoding ribosomal protein S21), dnaG (encoding DNA primase), and rpoD (encoding the principal $\sigma$ factor) (49). In the Gram-positive bacterium B. subtilis this operon apparently lacks rpsU but maintains dnaG (49). In contrast, the open reading frames preceding the S. aureus plaC gene do not have any similarity with dnaG or rpsU (26). The principal $\sigma$ factor genes of some other Gram-positive bacteria have recently been found to have a similar organization as the plaC gene of S. aureus. The hrdB genes of S. coelicolor and S. griseus and the sigA gene of B. lactofermentum are not associated with either dnaG or rpsU (45, 47, 50).

Of all Gram-positive organisms, the vegetative $\sigma$ factor from B. subtilis has been well characterized. Besides the strong similarity of the $\sigma^{SA}$ protein with the vegetative $\sigma$ factor of B. subtilis, several lines of evidence suggest that $\sigma^{SA}$ is the ve-
tative σ factor of S. aureus. To identify genes for the principal σ factors of various eubacteria, Tanaka et al. (51) performed DNA hybridization analysis with a synthetic oligonucleotide probe corresponding to the highly conserved amino acid sequence present within the "RpoD box" of primary σ factors (15). They were able to identify only one signal corresponding to a single gene for S. aureus. Consistent with the above results, Basheer and Iordanescu (26) detected only one homology region corresponding to the plcC sequence in the S. aureus chromosome by Southern analysis. A construct expressing a probe corresponding to the highly conserved amino acid sequence (25) was obtained only in cells carrying cloned plasmid vectors. In such cells, the maintenance of the vegetative state resulted convincingly prove that the plcC gene product is the vegetative σ factor.

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