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Coagulase Expression in *Staphylococcus aureus* Is Positively and Negatively Modulated by an *agr*-Dependent Mechanism

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Expression of staphylocoagulase by *agr*$^+$ *Staphylococcus aureus* depends on the growth phase, being maximal during exponential growth and decreasing sharply postexponentially, while an *agr*-deleted strain continuously expresses an intermediate level of coagulase. Therefore, coagulase expression appears to be both positively and negatively modulated by an *agr*-dependent mechanism.

Staphylocoagulase is an extracellular protein produced by *Staphylococcus aureus* which promotes fibrin formation in human plasma by nonenzymatic activation of prothrombin (14). It, like the cell wall-associated protein A, is mainly synthesized during exponential growth (3, 7). In contrast, other extracellular proteins and toxins (e.g., serine protease; nuclease; lipase; fibrinolysin; α-, β-, and δ-hemolysin; toxic shock syndrome toxin 1; and enterotoxin B) are produced after the end of exponential growth (1). The expression of these products is controlled by a complex regulatory locus, *agr* (6, 9, 10), whose unique feature is control of the expression of its target genes by means of an RNA molecule (RNAIII) (8). *agr* RNAIII acts as a positive regulator on genes which are preferentially expressed postexponentially, whereas protein A and coagulase are inhibited. The kinetics of protein A gene (*spa*) transcription has been investigated with *agr*$^+$ and *agr* mutant strains (12) and shown to be inversely related to the level of RNAIII. The proposed mechanism was that RNAIII had a strict inhibitory effect on *spa* expression. To our knowledge, no similar study of *agr* regulation of the coagulase gene (*coa*) has been conducted. Our results indicate that the regulation of *coa* expression is different from that of *spa*, in that coagulase expression may be modulated both positively and negatively by the *agr* system according to the growth stage.

Kinetics of coagulase activity. The coagulase activity of strain RN6390 (9), an *agr*$^+$ derivative of *S. aureus* 8325-4, was compared with that of RN6911, which was constructed by allelic replacement of the *agr* locus in RN6390 (8). Brain heart infusion broth (50 ml) in 500-ml Erlenmeyer flasks was inoculated with 10 colonies of overnight cultures on blood agar plates and incubated at 37°C with agitation at 190 rpm. Growth was monitored spectrophotometrically. Titration of coagulase activity was performed hourly for supernatants as described by McDevitt et al. (5) with Difco EDTA rabbit plasma. The titer was the reciprocal of the highest dilution of the supernatant showing evidence of clotting after 18 h of incubation at 37°C. Results were expressed as the ratio of titer to A_{540} at the time of sampling.

The two strains had similar latency times and growth rates under the cultivation conditions used in this study (Fig. 1). The *agr*$^+$ strain RN6390 displayed a maximum clotting activity of 18.6 after 2 to 3 h of growth (Fig. 1A), which decreased rapidly to become undetectable after 7 h. The *agr* mutant strain RN6911 had a relatively constant coagulase activity of 8 (8.57 ± 1.68) over the same period (Fig. 1B).

Kinetics of *coa* mRNA. The kinetics of *coa* mRNA expression was studied in the two strains by Northern (RNA) blot hybridization with a *coa*-specific RNA probe synthesized by in vitro transcription of pLUG12 (13) with digoxigenin-labeled UTP (Boehringer Mannheim) as substrate. A volume of culture corresponding to an A_{540} of 0.5 was removed hourly and used to prepare total RNAs as described previously (4). Crude extracts from the two strains were shown to contain the same amount of total RNA by electrophoresis of pure and serial twofold dilutions on the same 1% agarose gel and comparison of the intensities of ethidium bromide-stained 16S and 23S rRNA signals. Standardized RNA samples were electrophoresed through a 1% agarose gel containing 2.2 M formaldehyde and transferred to nylon membranes as described previously (12). Hybridization and detection of luminescence were carried out as described previously (13). Relative amounts of mRNA in the samples were estimated by comparison of signals at different dilutions.

A *coa* mRNA-specific signal was detected at 3 and 7 h in both strains (Fig. 2A). In RN6390, the relative amount of *coa* mRNA was four times higher at 3 h than at 7 h, while in RN6911 the level of *coa* mRNA was equivalent at both time points and corresponded to half the amount detected in RN6390 at 3 h and twice that at 7 h. These results complement those obtained by coagulase titration of supernatants from the same strains and show a direct relationship between the mRNA level and clotting activity in the supernatant. Coagulase expression by the colonies used for inoculation of the broth was not directly measured at time 0, but the *coa* mRNA measured in RN6390 at 3 h must represent newly synthesized molecules, because no *coa* mRNA signal was detectable at time 0 (Fig. 2C). RN6911, on the other hand, expressed *coa* mRNA at time 0, suggesting a constitutive expression of *coa* in the *agr* mutant background (Fig. 2C).

Inverse correlation between coagulase expression and RNAIII transcription. The kinetics of RNAIII in RN6390 was studied by Northern blot hybridization with an *agr* RNAIII

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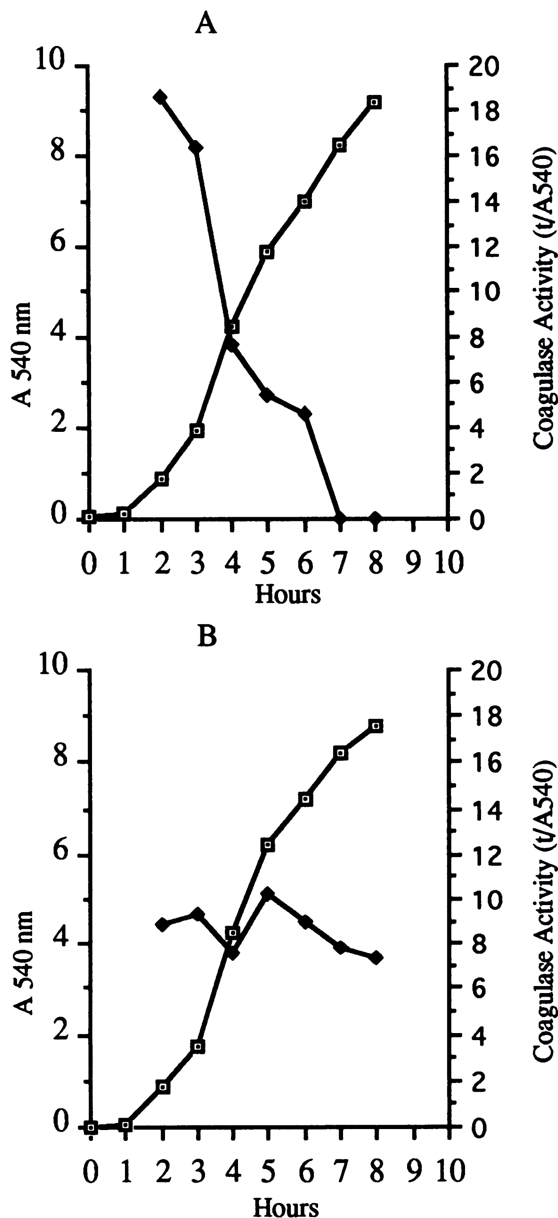

FIG. 1. Growth (□) and coagulase production (◆) by RN6390 (A) and RN6911 (B) grown in brain heart infusion broth with shaking at 37°C. Hourly samples were centrifuged, and the coagulase activity in the supernatant was assayed as described in the text. The results are expressed as the ratio of titer to A_{540} (t/A540) at the time of sampling.

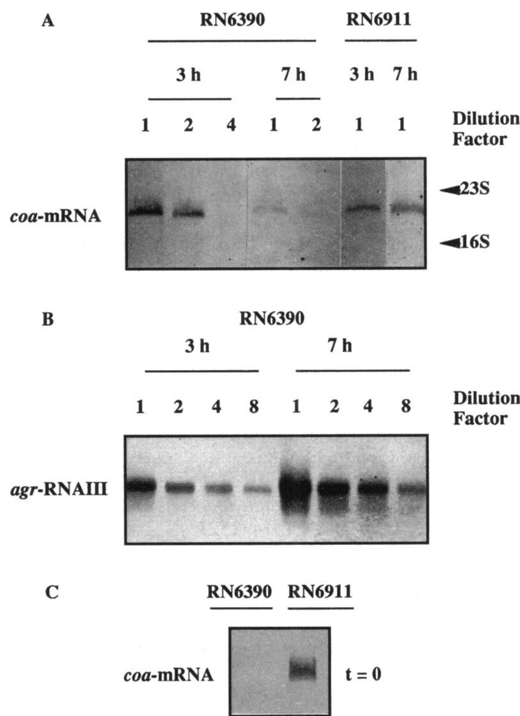

FIG. 2. Kinetics of *coa* mRNA from RN6390 and RN6911 (A), kinetics of *agr* RNAIII from RN6390 (B), and expression of *coa* mRNA in inoculum cells at a titer (t) of 0 (C) analyzed by Northern blot hybridization. Total cellular RNAs isolated at 0, 3, and 7 h of growth were electrophoresed through a 1% agarose gel containing 2.2 M formaldehyde, transferred to nylon membranes, and hybridized with digoxigenin-labeled RNA probes corresponding to the 871-bp *Eco*RI-*Hin*dIII fragment of *coa* (A and C) and the 962-bp *Cla*I-*Pst*I fragment of *agr* (B). Different samples with the same dilution factor contain the same amount of total RNA, except in panel C, in which total colony RNA was analyzed.

probe synthesized by in-vitro T3 polymerase transcription of a 962-bp *Cla*I-*Pst*I *agr* fragment subcloned from pRN6850 (9) to pBluescript II KS+/− (Stratagene). The results showed that the level of RNAIII varied inversely with that of *coa* mRNA, increasing by two to four times between 3 and 7 h of culture (Fig. 2B). This observation confirms the proposed inhibitory effect of *agr* on *coa* expression (6, 9) but does not explain the fact that the level of *coa* mRNA at the early time point is greater in the agr^{+} strain than in the *agr* mutant strain.

The regulation of coagulase expression appears therefore to be different from that of protein A. In RN6911, *spa* mRNA is constitutively expressed at a high level, while in RN6390 *spa* mRNA is transcribed at a lower level and disappears upon the appearance of RNAIII (12). For coagulase, our results show that the presence of a functional *agr* element results in a relative elevation of the *coa* mRNA level at the early time point followed by a diminution of this transcript at the later time point. Because the two isogenic strains studied differ only in their *agr* status, these results suggest that coagulase expression is both positively and negatively modulated by an *agr*-dependent mechanism.

Different hypotheses might be proposed to explain these observations. It has been suggested that RNAIII may interact with accessory protein factors to form a nucleoprotein complex (2, 8, 11). The small amount of RNAIII in early growth could be sufficient to stimulate a coagulase-activating factor whose positive effect would be masked later in the growth cycle, when RNAIII reaches a higher concentration. Alternatively, RNAIII could have a dual effect, depending on its secondary structure. A growth-dependent factor might induce a conformational change in native RNAIII, resulting in a reversal of effect (from activator to inhibitor) on the coagulase gene. This hypothesis is supported by the observation that RNAIII can have either stimulatory or inhibitory properties, depending on the target genes (8). Moreover, it has been shown that RNAIII has a dual effect on the *hla* gene, which seems to be controlled both at the level of transcription and at the level of translation by RNAIII (8).

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