

Transcription of *Staphylococcus aureus* Fibronectin Binding Protein Genes Is Negatively Regulated by *agr* and an *agr*-Independent Mechanism

PATRICIA SARA VIA-OTTEN,¹ HANS-PETER MÜLLER,^{2†} AND STAFFAN ARVIDSON^{1*}

Microbiology and Tumorbiology Center, Karolinska Institute, S-171 77 Stockholm,¹ and Department of Microbiology, Swedish University of Agricultural Sciences, S-750 07 Uppsala,² Sweden

Received 21 April 1997/Accepted 26 June 1997

The production of cell surface proteins in *Staphylococcus aureus* is generally down-regulated in the postexponential growth phase by the global regulator *agr*. The effector of this regulation is the RNAIII molecule, which is encoded within the *agr* locus. RNAIII seems to regulate most target genes at the level of transcription, but it also has an effect on the translation of some genes. To study the role of *agr* on the expression of fibronectin binding proteins (FnBPs), we investigated the transcription and translation of *fnb* genes in *agr* mutant strain WA250 and its parent strain, 8325-4. The results show that *fnb* genes are negatively regulated by *agr* and also by an *agr*-independent mechanism that restricts *fnb* mRNA synthesis to the early exponential phase of growth. Transcription and Western blot analysis of cell-associated FnBPs demonstrated that synthesis of both FnBPA and FnBPB in the wild-type and *agr* mutant strains took place preferentially during the first hour of growth and rapidly decreased after the second hour. We also confirmed previous results showing that the *agr* mutant strain has an increased capacity to bind fibronectin compared to its parent *agr*⁺ strain. However, while the concentrations of *fnb* mRNAs and proteins differed by a factor of 16 between the strains, the difference in fibronectin binding was only twofold, indicating that the binding of fibronectin to the bacteria is not proportional to the amount of FnBPs on their surface.

The initial step in an infectious disease is often adhesion to and colonization of host tissue surfaces. *Staphylococcus aureus*, which is a major human and animal pathogenic organism, has been shown to bind to several host matrix proteins and plasma proteins, such as fibronectin, fibrinogen, collagen (11), elastin (35), laminin (16, 41), prothrombin (4), thrombospondin (15), bone sialoprotein (37), and vitronectin (24, 25). For each of these binding functions, a corresponding surface-associated protein has been identified. The existence of an *S. aureus* extracellular matrix binding protein with broad specificity that is capable of binding several extracellular glycoproteins has also been reported (27). The role of some of these proteins in the pathogenesis of staphylococcal infections has been shown in animal models (10, 11, 28, 34).

Most *S. aureus* strains bind to fibronectin and two highly homologous fibronectin binding proteins (FnBPs), and their corresponding genes (*fnbA* and *fnbB*) have been identified (9, 12, 21). Mutants defective in either of the two genes adhered equally well to fibronectin-coated surfaces in vitro, while a double mutant was completely unable to adhere, indicating that both genes are expressed and contribute to fibronectin binding (13).

The ability of *S. aureus* cells to bind fibronectin is affected by two global regulatory systems, *agr* and *sar* (1, 3, 6, 7). Of these, *agr* is the best characterized and has been shown to act at the levels of both transcription and translation to regulate the production of many secreted toxins, enzymes, and cell surface proteins (29, 30, 36). Toxins and enzymes are generally posi-

tively controlled, while the production of cell surface proteins is negatively controlled (1, 18, 33). The effector of this positive and negative regulation is an RNA molecule, RNAIII (17, 30, 33), which is synthesized in response to the environmental concentration of an autocrine octapeptide synthesized by *agrB* and *agrD* (20). The octapeptide concentration is sensed by the *agrC* product, which resembles the signal sensor of classical two-component systems (20). AgrC is supposed to phosphorylate AgrA, which then activates transcription of the RNAIII-encoding gene. The *sar* locus, which appears to modulate the activity of *agr* (8, 14, 31), also seems to have a direct effect on fibronectin binding, which was decreased in *sar* mutants (3, 6, 7).

In several reports, *agr* mutants have been shown to bind more fibronectin than the corresponding wild-type strains (1, 6, 7). Considering the high sensitivity of FnBP to proteolytic degradation (13, 21), the increased fibronectin binding in the *agr* mutants could be due to decreased production of proteases rather than to increased synthesis of FnBPs.

To study the role of *agr* on the expression of FnBPs, we investigated the transcription and translation of *fnb* genes in an *agr* mutant and the corresponding wild-type strain. Our results show that the *fnb* genes are negatively regulated by *agr* and by an *agr*-independent mechanism that restricts FnBP synthesis to the early exponential phase of growth.

MATERIALS AND METHODS

Bacterial strains. *S. aureus* 8325-4 is our standard *agr*⁺ strain, which is a derivative of NTCC8325 cured of prophages (32). WA250, derived from 8325-4, has a Tn551 insertion in the *agrA* gene (29). RN6911 is a derivative of RN6390B in which the *agr* locus has been replaced with *tetM* (33). DU5883 is a derivative of 8325-4 in which the *fnbA* and *fnbB* genes have been inactivated by insertion mutagenesis (13). *Escherichia coli* MC1061 (5) was used as the host for plasmid constructions.

Growth conditions. *S. aureus* strains were routinely precultured in tryptic soy broth (TSB; Difco) for 16 to 18 h. Cells from 2 ml of preculture were transferred to 100 ml of brain heart infusion (BHI; Difco) in a 1-liter baffled flask and

* Corresponding author. Mailing address: Microbiology and Tumorbiology Center, Box 280, Karolinska Institute, S-171 77 Stockholm, Sweden. Phone: 46 8 7287172. Fax: 46 8 331547. E-mail: Staffan.Arvidson@mtc.ki.se.

† Present address: Ernst-Moritz-Arndt-Universität Institut für Immunologie und Transfusionsmedizin, Greifswald, Germany.

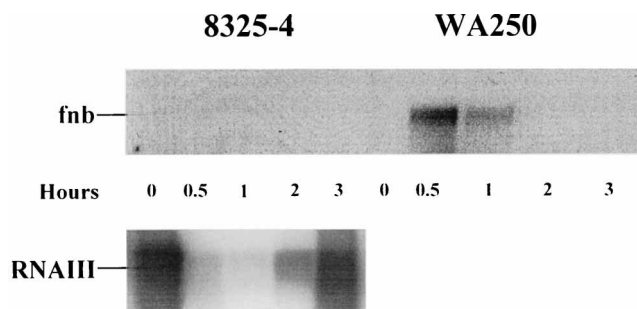


FIG. 1. Northern blot analysis of *fnb* transcripts and RNAIII in *S. aureus* 8325-4 (*agr*⁺) and the *agr* mutant WA250. Equal amounts of total cellular RNA isolated at the indicated time points during growth were analyzed by using specific antisense RNA probes as described in Materials and Methods.

incubated on a rotary shaker at 37°C. Cultivation for fibronectin binding assays was done with TSB (Difco) supplemented with 0.6% yeast extract (Difco). Bacterial growth was monitored by measuring optical density at 600 nm (OD₆₀₀). Wherever needed, antibiotics were added as follows: erythromycin, 10 µg ml⁻¹; tetracycline, 3 µg ml⁻¹.

Construction of plasmids and transformation. Competent *E. coli* cells were prepared and transformed as described by Sambrook et al. (38). Transformants were selected on Luria-Bertani plates containing 50 µg of ampicillin ml⁻¹. Chromosomal *S. aureus* DNA was prepared as described by Löfdahl et al. (26). Plasmid DNA was extracted by the alkaline lysis method (38) and further purified by using JetSorb (Genomed).

pEX107 contains an internal fragment of the RNAIII-encoding gene (nucleotides [nt] 1187 to 1565 in the published sequence [18]) in the antisense orientation behind the T3 promoter of pEX100 (30).

For pWA1, a 534-bp DNA fragment (nt 185 to 719 in the published sequence [39]) containing part of the *fnbA* gene was synthesized by PCR using the primers 5'-CAATGATCGTTGTTGGGATGGGAC-3' and 5'-ACTTACTTGTTACA TCTGTACC-3' with *S. aureus* 8325-4 chromosomal DNA as the template. The PCR product was cloned into pGEM-T (Promega). The orientation of the insert, assessed by restriction enzyme cleavage, was such that an *fnbA* antisense RNA was produced from the SP6 promoter of the plasmid.

In vitro synthesis of digoxigenin-labeled antisense RNA probes. pEX107 was used as the template for synthesis of an antisense RNAIII probe. The plasmid was linearized with *Eco*RI and transcribed by using T3 RNA polymerase as described by the supplier (DIG RNA Labeling Mix; Boehringer Mannheim). An antisense *fnbA* probe was synthesized from pWA1. The plasmid was digested with *Sph*I, and the protruding 3' termini generated were removed by using the Klenow fragment of *E. coli* DNA polymerase I. The antisense transcript was generated by SP6 polymerase as described above.

Northern blotting and primer extension analysis. Total *S. aureus* RNA was prepared as previously described (19). Northern blot analysis was performed as described by Sambrook et al. (38) by using positively charged nylon membranes (Boehringer Mannheim). Hybridization was carried out as described by the manufacturer (Boehringer Mannheim). Subsequently, the membranes were analyzed by chemiluminescence detection (DIG Luminescent Detection Kit; Boehringer Mannheim). Primer extension experiments were performed as previously described (31), by using 1 pmol of primer 5'-GTTTCTATATTGCTG GTTGTGCAG-3' (nt 428 to 453 [39]) for *fnbA* or 1 pmol of primer 5'-CGCG AAGTTTCTACTTTTGGTGC-3' (nt 820 to 842 [21]) for *fnbB* labeled with [γ -³²P]ATP (Amersham). Twenty micrograms of *S. aureus* total RNA was used as the template. Radioactivity was detected by a radioisotope imaging system (PhosphorImager 445SI; Molecular Dynamics). Northern blot and primer extension images were processed by using ImageQuant software.

Analysis of FnBPs by Western blotting. Cell-associated FnBPs were released by lysostaphin treatment of equal number of cells (OD₆₀₀ of 1.2) as previously described (19). Culture supernatants were dialyzed against water and concentrated 100-fold by lyophilization. Samples were separated by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) with 7.5% acrylamide resolving gels as previously described (22). After being transferred to nitrocellulose membranes (Schleicher & Schuell), FnBPs were detected with goat anti-serum against the fibronectin binding D domains of FnBPA from *S. aureus* (gift from L. K. Rantamäki, University of Helsinki, Helsinki, Finland). For development, rabbit anti-goat peroxidase-conjugated antibody (DAKO) was used as recommended by the supplier.

Assay for fibronectin binding. Binding of fibronectin to *S. aureus* cells was measured as described earlier (9). Washed *S. aureus* cells (10⁹ CFU) harvested at different times during growth were incubated with the [¹²⁵I]-labeled (90,000 cpm) 24-kDa amino-terminal fragment of bovine fibronectin (21) in phosphate-buffered saline (PBS) containing 0.1% Tween 20 in a total volume of 0.3 ml.

After incubation for 2 h at room temperature, the radioactivity bound to the cells was measured in a gamma counter. All assays were done in duplicate.

RESULTS

Effect of *agr* on *fnb* transcription. Transcription of the *fnb* genes in strain 8325-4 and the *agr* mutant WA250 was analyzed by Northern blotting. Samples for RNA analysis were taken at intervals during the exponential and postexponential phases of growth. In numerous experiments using different culture media (BHI, TSB, and casein hydrolysate-yeast extract with and without glucose), *fnb* mRNA was detected only in the *agr* mutant (Fig. 1), suggesting that transcription of the *fnb* genes is negatively regulated by *agr*. Analysis of twofold serial dilutions of total RNA from both strains revealed that the *agr* mutant produced at least 16 times as much *fnb* mRNA as the wild-type strain (data not shown). Unlike that of other negatively regulated genes (i.e., *spa* and *coa*) which are constitutively expressed in *agr* mutants (23, 40), transcription of *fnb* was restricted to the first hour of growth (lag phase and first cell division).

As expected, no RNAIII was detected in the *agr* mutant (WA250) throughout the growth experiment (data not shown), indicating that the down-regulation of *fnb* expression at the beginning of the exponential growth phase was due to an *agr*-independent mechanism. This was confirmed by the observation of the same pattern of *fnb* expression in another *agr* mutant, RN6911, lacking the entire *agr* locus, including the RNAIII gene. In wild-type cells, large amounts of RNAIII were present at zero time; these decreased dramatically during the first 30 min of growth and then increased again after 2 to 3 h (Fig. 1). The presence of RNAIII during the lag phase and the early exponential phase of growth thus seemed to repress the transcription of *fnb* genes completely.

When wild-type bacteria were precultured in BHI instead of TSB, much less RNAIII was present at zero time and the level decreased below the detection limit within the first hour of growth in fresh BHI medium. Under these conditions, significant amounts of *fnb* mRNAs were found in the 1-h sample (see lane 0 in Fig. 2). When these cells, without any detectable RNAIII, were used to inoculate fresh BHI medium, there was no further increase in *fnb* mRNAs, in spite of the fact that RNAIII remained below the detection limit until 2 h (Fig. 2). Essentially the same result was obtained with *agr* mutant strain WA250 (data not shown). Together, these results show that transcription of the *fnb* genes is restricted to the first hour of growth (1 to 2 cell divisions) immediately after the bacteria have been transferred from a stationary-phase culture to fresh

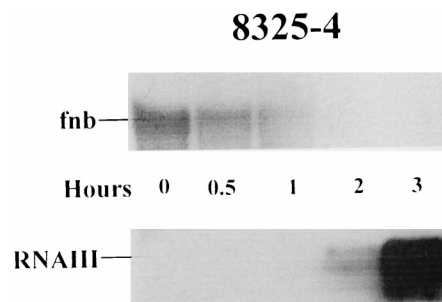


FIG. 2. Northern blot analysis of *fnb* transcripts and RNAIII in *S. aureus* 8325-4. Cells depleted of RNAIII by overnight cultivation in BHI, followed by 1 h in fresh BHI (zero hour sample), were used to inoculate fresh BHI medium. Total cellular RNA was isolated at the indicated time points and analyzed by using specific antisense RNA probes as described in Materials and Methods.

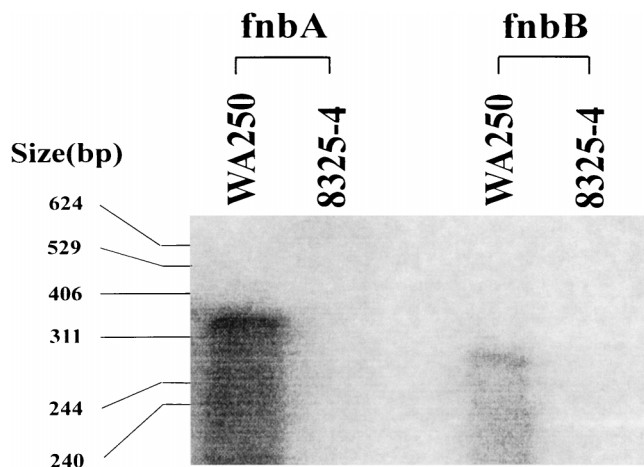


FIG. 3. Primer extension analysis of *fnbA* and *fnbB* mRNAs in *S. aureus* 8325-4 (*agr*⁺) and the *agr* mutant WA250. Twenty micrograms of total RNA was used as the template for primer extension reactions with specific primers for the *fnbA* and *fnbB* genes, respectively. Shown is the analysis of RNA samples taken after 1 h of growth in BHI. Molecular size markers are indicated.

medium and that this temporal regulation is independent of *agr*.

Primer extension experiments were carried out to verify the Northern blot results and to check that both *fnb* genes were expressed. Based on the differences between the nucleotide sequences in the 5' end of the *fnb* genes (21), two nucleotide primers were designed to anneal specifically to the *fnbA* and *fnbB* mRNAs, respectively. Product lengths were calculated according to the reported locations of the transcription start points (13) as 402 nt for *fnbA* and 389 nt for *fnbB*. As shown in Fig. 3, products of the expected sizes were obtained when total RNA from the *agr* mutant was used as the template while no products were obtained with equal amounts of wild-type RNA. As for the Northern blot analysis, *fnb* transcripts were detected only during the first hour in the mutant strain. This confirmed that both genes were expressed and that they were negatively regulated by *agr*.

Analysis of cell-associated FnBPs in *agr*⁺ and *agr* mutant strains. Production of cell-associated FnBPs during the exponential and postexponential phases of growth was assessed by Western (immunoblotting) analysis of lysostaphin extracts from wild-type and *agr* mutant cells. The antibodies used for detection were directed against the fibronectin binding do-

main (D1 to D3), which are 95% identical in FnBPA and FnBPB. Two major proteins with apparent molecular masses of around 200 kDa were seen in extracts from both the wild type and the *agr* mutant (Fig. 4), which is in agreement with the reported values for the native forms of FnBPA and FnBPB (12, 13, 21). Several smaller bands of variable sizes which reacted with the antibodies probably represent degradation products of the FnBPs, as they were not seen in extracts from a mutant lacking both FnBPA and FnBPB (DU5883) (Fig. 4, line C). Analysis of twofold dilution series of extracts from the *agr* mutant revealed that the amount of FnBPs was more than 16 times higher than that in the wild-type strain, which is in agreement with the difference in *fnb* mRNA concentration between the strains.

As seen in Fig. 4, de novo synthesis of FnBPs took place preferentially during the first hour of growth, which is consistent with the transcription analysis (Fig. 1 and 2). Full-length FnBPs decreased dramatically between 2 and 3 h and were not seen in extracts from bacteria taken after 16 h of growth (Fig. 4, lane zero samples represent preculture cells grown for 16 h). The nature of the smaller bands seen in time zero samples is not known.

As only trace amounts of FnBPs, or their degradation products, were found in culture supernatants from the *agr* mutant during the exponential phase of growth (data not shown), we conclude that the majority of FnBPs were associated with the bacterial cells. No proteins were detected by the anti-FnBP serum in supernatants from the wild-type strain during the exponential phase of growth.

Binding of fibronectin to *agr*⁺ and *agr* mutant strains. Previous studies have shown that stationary-phase cells of *agr* mutants bind 0.5 to 4 times as much fibronectin as do those of the corresponding wild-type strain (1, 6, 7). To correlate fibronectin binding with our present data on the expression of the *fnb* genes, wild-type and *agr* mutant cells, harvested at hourly intervals during the early phase of growth, were incubated with radiolabeled fibronectin as described in Materials and Methods. As shown in Fig. 5, fibronectin binding increased significantly during the first hour of growth in both strains, the mutant reaching twice the level of the wild-type strain. This was consistent with the simultaneous increase in the expression of the *fnb* genes and suggests that the fibronectin binding was mediated by the de novo-synthesized FnBPs. However, while the concentrations of *fnb* mRNAs and proteins differed between the strains by a factor of 16 the difference in fibronectin binding was only twofold, indicating that the in vitro binding of

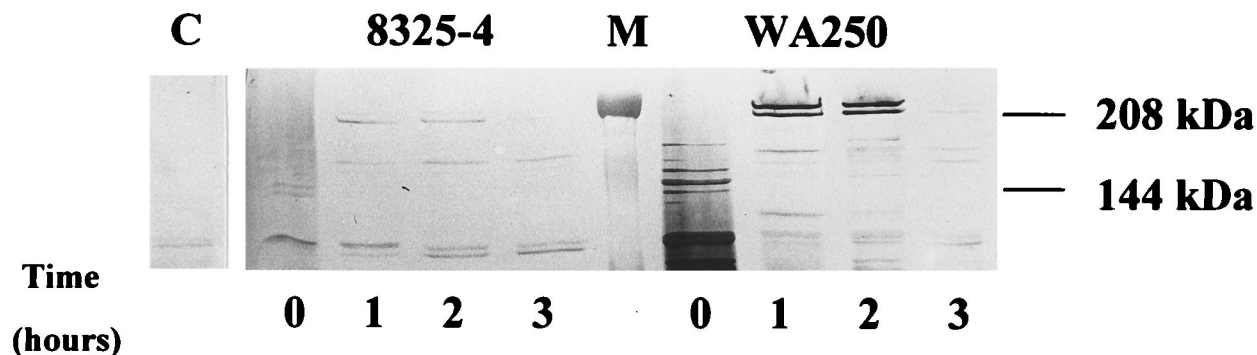


FIG. 4. Western blot analysis of cell-associated FnBP released by lysostaphin treatment of equivalent numbers of cells of *S. aureus* 8325-4 (*agr*⁺) and WA250 (*agr* mutant) taken at the indicated time points. Extract from the double *fnbA fnbB* mutant strain DU5883 was used as a negative control (C). Sizes of marker proteins (Kaleidoscope prestained standards; Bio-Rad) in lane M are indicated.

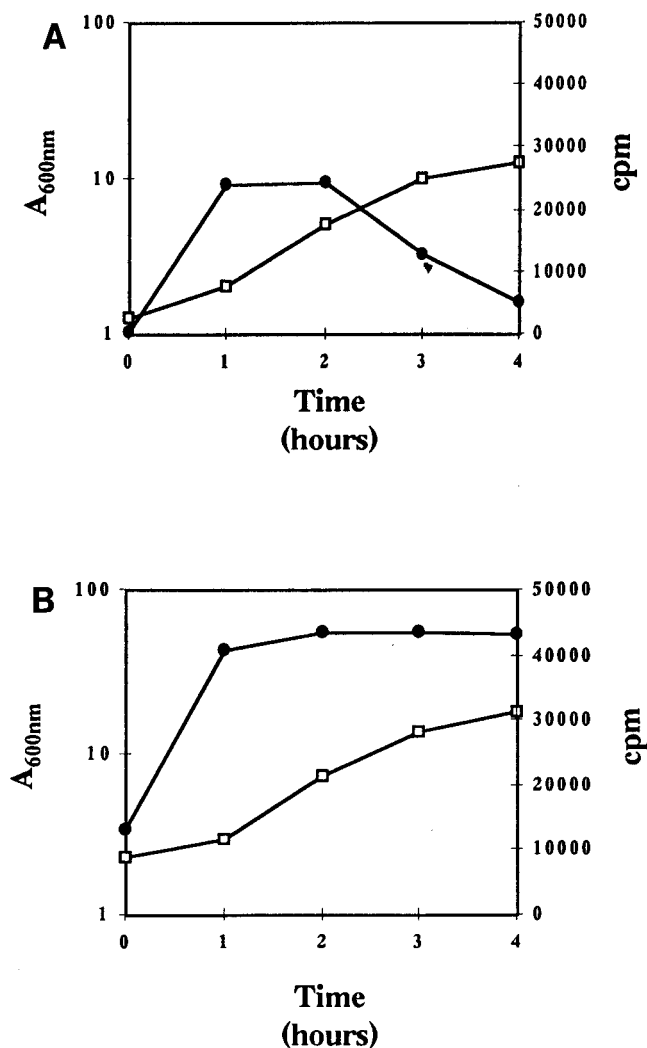


FIG. 5. Binding of ^{125}I -labeled fibronectin (●) to *S. aureus* 8325-4 (A) and WA250 (B) during growth (□) in TSB plus yeast extract. The binding of radio-labeled fibronectin to 10^9 CFU of bacteria was measured. Counts per minute are the means of two independent measurements. Bacterial growth was monitored by measuring OD₆₀₀.

fibronectin to the bacteria is not proportional to the amount of FnBPs on their surface.

DISCUSSION

In the present study, we have shown that the transcription of *fnb* genes in *S. aureus* is negatively controlled by *agr* and also by another, *agr*-independent, mechanism. Under the conditions used, *fnbA* and *fnbB* seem to be coordinately regulated, which is consistent with previous studies using *fnb* promoter fusions (13). It has been shown earlier that transcription of the genes coding for protein A (*spa*) and coagulase (*coa*) are also negatively regulated by *agr* and that the effector of this regulation is RNAIII (23, 40). In *agr* mutants, lacking RNAIII, *spa* and *coa* are therefore transcribed throughout the exponential and post-exponential phases of growth (23, 40). In contrast, synthesis of *fnb* mRNA in two different *agr* mutants (WA250 and RN6911) was restricted to the first hour of growth, corresponding to the lag phase and the first cell division. Since RN6911 lacks the whole *agr* locus, including the RNAIII gene, it can be con-

cluded that this temporal expression of the *fnb* genes must be due to *agr*-independent regulation.

According to the general model for the *agr* system, genes that are negatively regulated by *agr* should be expressed in wild-type strains during the early exponential phase of growth, before RNAIII starts to accumulate (17, 29, 36). This has also been demonstrated for *spa* and *coa* (23, 40). However, since *fnb* expression was restricted to the first hour of growth and high concentrations of RNA III were present in the wild-type bacteria at the beginning of this period of time, only trace amounts of *fnb* mRNA could be detected. Although the concentration of RNAIII decreased rapidly during the first 30 min of growth, trace amounts were still present after 1 h (Fig. 1). This is consistent with an RNAIII half-life of 15 min (2). Our results also indicate that transcription of the *fnb* genes is much more sensitive to RNAIII repression than is that of *coa*, which was expressed even at relatively high RNAIII concentrations (23).

In wild-type cultures with less initial RNAIII (precultured in BHI), the concentration of RNAIII decreased below the detection limit within the first hour of growth and allowed some transcription of *fnb* to occur. However, transcription again seemed to be restricted to the first hour of growth although RNAIII was not detected. After 1 h of growth, *fnb* transcription did not continue even when the bacteria were shifted into fresh medium (Fig. 2; data not shown for the *agr* mutant). This suggests that it is not some factor in the fresh culture medium that stimulates transcription of the *fnb* genes during the early exponential phase of growth, but rather it is the metabolic status of the bacterial cells that determines whether transcription takes place or not. It thus seems that stationary-phase bacteria which are transferred to fresh medium become competent for *fnb* transcription while cells that have been growing exponentially for 1 h do not.

The rapid increase in cell-associated FnBPs and fibronectin binding during the first hour of growth was consistent with the results of the mRNA analysis in both wild-type and *agr* mutant cells. However, while the *fnb* mRNA or FnBP levels differed by a factor of 16 between the strains the difference in fibronectin binding was only twofold. A possible explanation for this discrepancy is that other cell surface components that are up-regulated in the *agr* mutants interfere with fibronectin binding or that binding is simply not proportional to the number of FnBPs on the bacterial cells because of sterical reasons.

In summary, the present results show that expression of the *fnb* genes in *S. aureus* is negatively regulated by *agr*, and by an *agr*-independent mechanism, in such a way that synthesis of FnBPs is restricted to a short period of time in the early exponential phase of growth. This means that the ability of *S. aureus* cells to adhere to fibronectin in vivo probably changes dramatically during the course of infection. To fully understand the role of fibronectin binding in the infection process, the expression of *agr* and the *fnb* genes needs to be studied in vivo.

ACKNOWLEDGMENTS

We thank Agneta Wahlquist for skillful technical assistance. We are also grateful to Esteban Chaves-Olarte for invaluable help with the preparation of the manuscript.

This work was supported by the Swedish Agency for Research Cooperation with Developing Countries (SAREC) and by grant 4513 from the Swedish Medical Research Council. P. Saravia-Otten was a participant in the Karolinska Institute International Research Training (KIRT) program.

REFERENCES

- Abdelnour, A., S. Arvidson, T. Bremell, C. Ryden, and A. Tarkowski. 1993. The accessory gene regulator (*agr*) controls *Staphylococcus aureus* virulence in a murine arthritis model. *Infect. Immun.* **61**:3879–3885.
- Arvidson, S., L. Janzon, S. Lofdahl, and E. Morfeldt. 1989. The exoprotein regulatory region (*exp*) of *Staphylococcus aureus*, p. 511–518. In L. O. Butler, C. Harwood, and B. E. B. Moseley (ed.), *Genetic transformation and expression*. Intercept Ltd., Winborn, Dorset, United Kingdom.
- Bayer, M. G., J. H. Heinrichs, and A. L. Cheung. 1996. The molecular architecture of the *sar* locus in *Staphylococcus aureus*. *J. Bacteriol.* **178**:4563–4570.
- Boden, M. K., and J. I. Flock. 1992. Evidence for three different fibrinogen-binding proteins with unique properties from *Staphylococcus aureus* strain Newman. *Microb. Pathog.* **12**:289–298.
- Casadaban, M., and S. N. Cohen. 1980. Analysis of gene control signals by DNA fusion and cloning in *Escherichia coli*. *J. Mol. Biol.* **138**:179–207.
- Cheung, A. L., K. J. Eberhardt, E. Chung, M. R. Yeaman, P. M. Sullam, M. Ramos, and A. S. Bayer. 1994. Diminished virulence of a *sar*⁻*agr*⁻ mutant of *Staphylococcus aureus* in the rabbit model of endocarditis. *J. Clin. Invest.* **94**:1815–1822.
- Cheung, A. L., J. M. Koomey, C. A. Butler, S. J. Projan, and V. A. Fischetti. 1992. Regulation of exoprotein expression in *Staphylococcus aureus* by a locus (*sar*) distinct from *agr*. *Proc. Natl. Acad. Sci. USA* **89**:6462–6466.
- Cheung, A. L., and S. J. Projan. 1994. Cloning and sequencing of *sarA* of *Staphylococcus aureus*, a gene required for the expression of *agr*. *J. Bacteriol.* **176**:4168–4172.
- Flock, J. I., G. Froman, K. Jonsson, B. Guss, C. Signas, B. Nilsson, G. Raucci, M. Hook, T. Wadstrom, and M. Lindberg. 1987. Cloning and expression of the gene for a fibronectin-binding protein from *Staphylococcus aureus*. *EMBO J.* **6**:2351–2357.
- Flock, J. I., S. A. Hienz, A. Heimdahl, and T. Schennings. 1996. Reconsideration of the role of fibronectin binding in endocarditis caused by *Staphylococcus aureus*. *Infect. Immun.* **64**:1876–1878.
- Foster, T. J., and D. McDevitt. 1994. Surface-associated proteins of *Staphylococcus aureus*: their possible roles in virulence. *FEMS Microbiol. Lett.* **118**:199–205.
- Froman, G., L. M. Switalski, P. Speziale, and M. Hook. 1987. Isolation and characterization of a fibronectin receptor from *Staphylococcus aureus*. *J. Biol. Chem.* **262**:6564–6571.
- Greene, C., D. McDevitt, P. Francois, P. E. Vaudaux, D. P. Lew, and T. J. Foster. 1995. Adhesion properties of mutants of *Staphylococcus aureus* defective in fibronectin-binding proteins and studies on the expression of *fnb* genes. *Mol. Microbiol.* **17**:1143–1152.
- Heinrichs, J. H., M. G. Bayer, and A. L. Cheung. 1996. Characterization of the *sar* locus and its interaction with *agr* in *Staphylococcus aureus*. *J. Bacteriol.* **178**:418–423.
- Herrmann, M., S. Suchard, L. Boxer, F. Waldvogel, and P. Lew. 1991. Thrombospondin binds to *Staphylococcus aureus* and promotes staphylococcal adherence to surfaces. *Infect. Immun.* **59**:279–288.
- Herrmann, M., P. E. Vaudaux, D. Pittet, R. Auckenthaler, P. D. Lew, F. Schumacher-Pedreau, G. Peters, and F. A. Waldvogel. 1988. Fibronectin, fibrinogen, and laminin act as mediators of adherence of clinical staphylococcal isolates to foreign material. *J. Infect. Dis.* **158**:693–701.
- Janzon, L., and S. Arvidson. 1990. The role of the delta-lysin gene (*hld*) in the regulation of virulence genes by the accessory gene regulator (*agr*) in *Staphylococcus aureus*. *EMBO J.* **9**:1391–1399.
- Janzon, L., S. Lofdahl, and S. Arvidson. 1989. Identification and nucleotide sequence of the delta-lysin gene, *hld*, adjacent to the accessory gene regulator (*agr*) of *Staphylococcus aureus*. *Mol. Gen. Genet.* **219**:480–485.
- Janzon, L., S. Lofdahl, and S. Arvidson. 1986. Evidence for a coordinate control of alpha-toxin and protein A in *Staphylococcus aureus*. *FEMS Microbiol. Lett.* **33**:193–198.
- Ji, G., R. C. Beavis, and R. P. Novick. 1995. Cell density control of staphylococcal virulence mediated by an octapeptide pheromone. *Proc. Natl. Acad. Sci. USA* **92**:12055–12059.
- Jonsson, K., C. Signas, H. P. Muller, and M. Lindberg. 1991. Two different genes encode fibronectin binding proteins in *Staphylococcus aureus*. The complete nucleotide sequence and characterization of the second gene. *Eur. J. Biochem.* **202**:1041–1048.
- Laemmli, U. K. 1970. Cleavage of structural proteins during the assembly of the head of bacteriophage T4. *Nature* **227**:680–685.
- Lebeau, C., F. Vandenesch, T. Greenland, R. P. Novick, and J. Etienne. 1994. Coagulase expression in *Staphylococcus aureus* is positively and negatively modulated by an *agr*-dependent mechanism. *J. Bacteriol.* **176**:5534–5536.
- Liang, O. D., J. I. Flock, and T. Wadstrom. 1995. Isolation and characterization of a vitronectin-binding surface protein from *Staphylococcus aureus*. *Biochim. Biophys. Acta* **1250**:110–116.
- Liang, O. D., M. Maccarana, J. I. Flock, M. Paulsson, K. T. Preissner, and T. Wadstrom. 1993. Multiple interactions between human vitronectin and *Staphylococcus aureus*. *Biochim. Biophys. Acta* **1225**:57–63.
- Lofdahl, S., B. Guss, M. Uhlen, L. Philipson, and M. Lindberg. 1983. Gene for staphylococcal protein A. *Proc. Natl. Acad. Sci. USA* **80**:697–701.
- McGavin, M. H., D. Krajewska-Pietrasik, C. Ryden, and M. Hook. 1993. Identification of a *Staphylococcus aureus* extracellular matrix-binding protein with broad specificity. *Infect. Immun.* **61**:2479–2485.
- Moreillon, P., J. M. Entenza, P. Francioli, D. McDevitt, T. J. Foster, P. Francois, and P. Vaudaux. 1995. Role of *Staphylococcus aureus* coagulase and clumping factor in pathogenesis of experimental endocarditis. *Infect. Immun.* **63**:4738–4743.
- Morfeldt, E., L. Janzon, S. Arvidson, and S. Lofdahl. 1988. Cloning of a chromosomal locus (*exp*) which regulates the expression of several exoprotein genes in *Staphylococcus aureus*. *Mol. Gen. Genet.* **211**:435–440.
- Morfeldt, E., D. Taylor, A. von Gabain, and S. Arvidson. 1995. Activation of alpha-toxin translation in *Staphylococcus aureus* by the trans-encoded anti-sense RNA, RNAlII. *EMBO J.* **14**:4569–4577.
- Morfeldt, E., K. Tegmark, and S. Arvidson. 1996. Transcriptional control of the *agr*-dependent virulence gene regulator, RNAlII, in *Staphylococcus aureus*. *Mol. Microbiol.* **21**:1227–1237.
- Novick, R. 1967. Properties of a cryptic high-frequency transducing phage in *Staphylococcus aureus*. *Virology* **33**:155–166.
- Novick, R. P., H. F. Ross, S. J. Projan, J. Kornblum, B. Kreiswirth, and S. Moghazeh. 1993. Synthesis of staphylococcal virulence factors is controlled by a regulatory RNA molecule. *EMBO J.* **12**:3967–3975.
- Palma, M., S. Nozohoor, T. Schennings, A. Heimdahl, and J.-I. Flock. 1996. Lack of the extracellular 19-kilodalton fibrinogen-binding protein from *Staphylococcus aureus* decreases virulence in experimental wound infection. *Infect. Immun.* **64**:5284–5289.
- Park, P., D. Roberts, L. Grosso, W. Parks, J. Rosenbloom, W. Abrams, and R. Mecham. 1991. Binding of elastin to *Staphylococcus aureus*. *J. Biol. Chem.* **266**:23399–23406.
- Reese, P., B. Kreiswirth, M. O'Reilly, P. Schlievert, A. Gruss, and R. P. Novick. 1986. Regulation of exoprotein gene expression in *Staphylococcus aureus* by *agr*. *Mol. Gen. Genet.* **202**:58–61.
- Ryden, C., A. I. Yacoub, I. Maxe, D. Heinegard, A. Oldberg, A. Franzen, A. Ljungh, and K. Rubin. 1989. Specific binding of bone sialoprotein to *Staphylococcus aureus* isolated from patients with osteomyelitis. *Eur. J. Biochem.* **184**:331–336.
- Sambrook, J., E. F. Fritsch, and T. Maniatis. 1989. *Molecular cloning: a laboratory manual*, 2nd ed. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, N.Y.
- Signas, C., G. Raucci, K. Jonsson, P. E. Lindgren, G. M. Anantharamaiah, M. Hook, and M. Lindberg. 1989. Nucleotide sequence of the gene for a fibronectin-binding protein from *Staphylococcus aureus*: use of this peptide sequence in the synthesis of biologically active peptides. *Proc. Natl. Acad. Sci. USA* **86**:699–703.
- Vandenesch, F., J. Kornblum, and R. P. Novick. 1991. A temporal signal, independent of *agr*, is required for *hla* but not *spa* transcription in *Staphylococcus aureus*. *J. Bacteriol.* **173**:6313–6320.
- Vercellotti, G., J. McCarthy, P. Lindholm, P. Peterson, H. Jacobs, and L. Furcht. 1985. Extracellular matrix proteins (fibronectin, laminin, and type IV collagen) bind and aggregate bacteria. *Am. J. Pathol.* **120**:13–21.