JOURNAL OF BACTERIOLOGY, Jan. 2008, p. 447–451 Vol. 190, No. 1
0021-9193/08/$08.00+0 doi:10.1128/JB.01174-07

Mapping the Transcription Start Points of the *Staphylococcus aureus* *eap*, *emp*, and *vwb* Promoters Reveals a Conserved Octanucleotide Sequence That Is Essential for Expression of These Genes▽†

Niamh Harraghy,[1]* Dagmar Homerova,[2] Mathias Herrmann,[1] and Jan Kormanec[2]

Institute of Medical Microbiology and Hygiene, University of Saarland, 66421 Homburg/Saar, Germany,[1] and Institute of Molecular Biology, Slovak Academy of Sciences, 84551 Bratislava, Slovakia[2]

Received 25 July 2007/Accepted 19 October 2007

Mapping the transcription start points of the *eap*, *emp*, and *vwb* promoters revealed a conserved octanucleotide sequence (COS). Deleting this sequence abolished the expression of *eap*, *emp*, and *vwb*. However, electrophoretic mobility shift assays gave no evidence that this sequence was a binding site for SarA or SaeR, known regulators of *eap* and *emp*.

The ability of *Staphylococcus aureus* to cause such diverse infections as endocarditis, pneumonia, skin infections, and biofilms is linked to its great repertoire of virulence factors, including adhesins, immunomodulatory molecules, and toxins (31). The *S. aureus* cell surface adhesins belong to one of two groups, the MSCRAMMs (microbial surface components recognizing adhesive matrix molecules), which include protein A, clumping factors A and B, and the fibronectin-binding proteins; and the SERAMs (secretable expanded repertoire adhesive molecules), which include the extracellular adherence protein (Eap), the extracellular matrix binding protein (Emp), and the extracellular fibrinogen-binding protein (Efb) (reviewed in references 5 and 7). The MSCRAMMs contain an LPXTG motif, which is involved in anchoring them to the staphylococcal cell surface (10, 32, 45), while the SERAMs lack this motif and may bind to the staphylococcal cell surface either covalently or via specific cell surface receptors (e.g., see references 11, 25, and 35). Together the MSCRAMMs and SERAMs facilitate the attachment of *S. aureus* to eukaryotic cells, platelets, extracellular matrix proteins, and inert surfaces (reviewed in reference 43) and may aid the survival and persistence of the staphylococci in the host due to their ability to interfere with the host's immune response (1, 4, 15, 22, 28, 30, 41, 44, 47).

Eap, Emp, and von Willebrand factor-binding protein (vWbp) are members of the SERAM family (5). While Eap is functionally well characterized (18), less is known about Emp and vWbp. Emp was described as an extracellular matrix binding protein, but additional functional roles have not yet been described (23). vWbp was identified during a screen for factors binding von Willebrand factor and was subsequently shown to be a coagulase (2, 3). Although the members of the SERAM family do not share significant sequence homology, they are recognized as sharing similar functional properties, such as being important in adhesion and modulation of the host immune response to staphylococcal infections (1–3, 5, 12, 16, 17, 23, 29, 30, 42, 44, 47). What is not yet known, however, is whether the regulation of the SERAMs at the molecular level is governed by a common mechanism or factor.

We became interested in studying the regulation of *emp* and *vwb* as these two genes, together with *clfA*, are located adjacent to each other (*S. aureus* strain COL open reading frames SACOL0856-SACOL0858), with 223 bp separating *clfA* from *vwb* and 353 bp separating *vwb* from *emp*. As ClfA is also an important *S. aureus* virulence factor, an interesting scenario would be the cotranscription of *clfA*, *vwb*, and *emp*. We mapped the transcription start points of *emp* and *vwb* in *S. aureus* strain Newman using primer extension analysis as described in reference 19 and found that both genes had their own promoter (Fig. 1A). These findings suggest that the genes are not cotranscribed and fit with the observations by us and others that the expression profiles of the three genes are different (3, 19, 34, 46). *eap* was also found to be transcribed from a single promoter (Fig. 1A). Putative promoter elements were identified by analysis of the region upstream of the transcription start point. All three promoters have a conserved −10 box (Fig. 1B), but homology to the consensus −35 box, TTGACA (20, 33, 36, 37), is less conserved, particularly in the *vwb* promoter. However, we have found a *c*onserved *o*ctanucleotide *s*equence (COS), AGTTAATT, that is just 5′ to a putative −35 box in each promoter (Fig. 1B). Moreover, searching the *S. aureus* COL genome for this COS revealed a COS in the same position (i.e., immediately upstream of a putative −35 box) in the promoters of several important virulence factors (Table 1). A common feature of these virulence factors is that they are involved in modulating the immune response to *S. aureus* infections or antibiotic resistance (5, 9, 24, 48). Taken together, these data suggested that the COS could be important in the regulation of these genes.

To investigate the importance of the COS, we deleted it in the *eap*, *emp*, and *vwb* promoters in a two-step PCR. For deleting the COS in the *emp* promoter, two primer pairs were used. Primers emp-cs_R (5′-GTTTACTTCAATTATACTGA AAATTC-3′) and emp-cs_F (5′-GAATTTTCAGTATAATT

* Corresponding author. Present address: Institute of Biotechnology UNIL, EPFL-FSB LBTM (Bâtiment CH), Station 6, CH-1015 Lausanne, Switzerland. Phone: 41 21 693 76 16. Fax: 41 21 693 76 10. E-mail: niamh.harraghy@unil.ch.

† Supplemental material for this article may be found at http://jb.asm.org/.

▽ Published ahead of print on 26 October 2007.

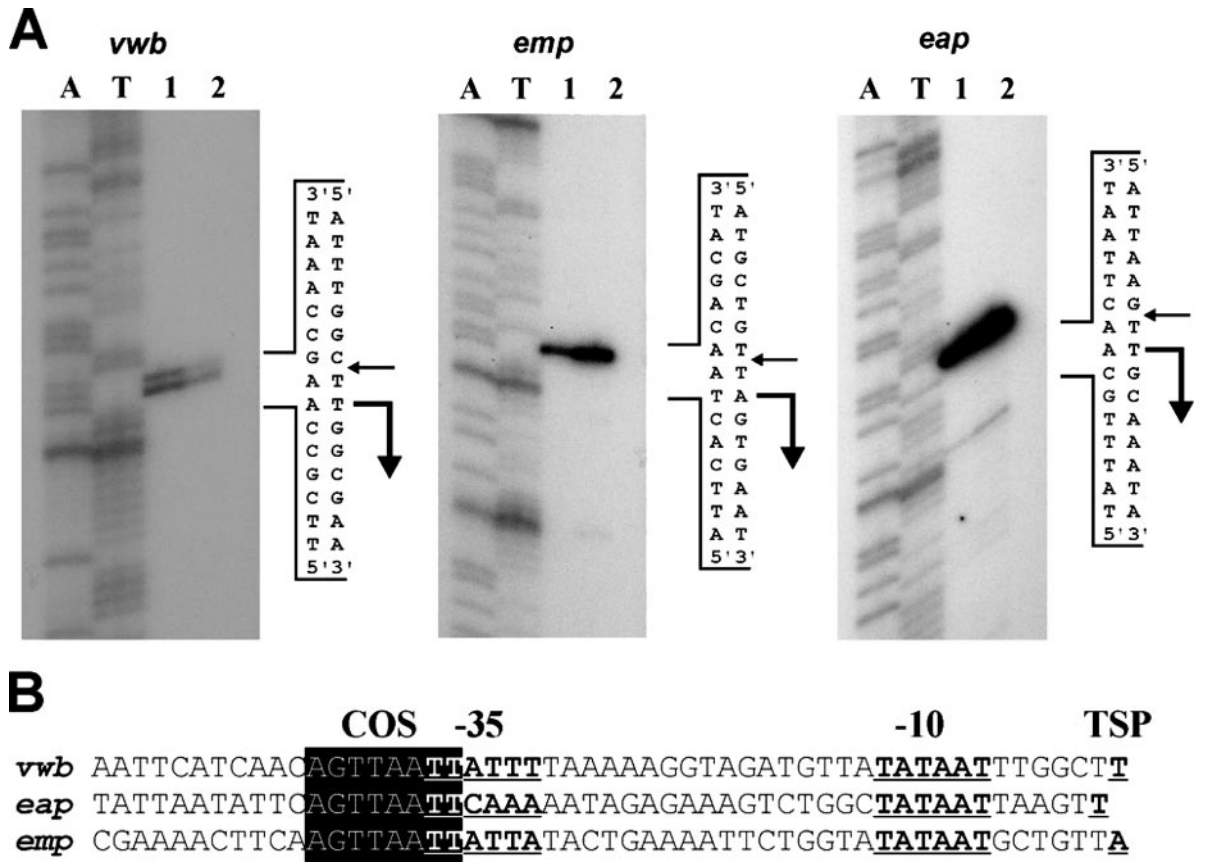

FIG. 1. Mapping the transcription start points (TSP) of the *emp*, *vwb*, and *eap* promoters (modified from Harraghy et al. [19], with permission of the publisher). (A) The TSP of the *vwb*, *emp*, and *eap* promoters were mapped by primer extension analysis as described in reference 19. (B) Putative −35 and −10 elements were identified by visual inspection of the region upstream of the TSP and are shown in bold. The COS is highlighted.

GAAGTAAAC-3′) are complementary and lack the COS. Primers empPF1 and empPR1 were described previously (19). In the first PCR, primers empPF1 and emp-cs_F were used to amplify the region 5′ of the COS, while primers empPR1 and emp-cs_R amplified the region 3′ of the COS. In the second PCR, the two PCR products were joined together using primers empPF1 and empPR1. For deleting the COS in the *vwb* promoter, primers vwbPF1 (5′-TTCGAATTCAGATAGCGA

TABLE 1. Mapping the COS in selected promoters relative to their putative −35 and −10 boxes

Open reading frame IDs for strains COL and N315	Gene	Sequence[a]
SACOL2002, SA1751	*eap*[b]	taatattc**AGTTAA*TT*caaa**aaatagagaaagtctggctataattaagt**t** N_{36} ATG
SACOL0858, SA0744	*emp*[b]	ttacttca**AGTTAA*TT*atta**tactgaaaattctggtatataatgctgtt**a** N_{63} ATG
SACOL0857, SA0743	*vwb*	tcatcaac**AGTTAA*TT*attt**taaaaaggtagatgttatataatttggct**t** N_{60} ATG
SACOL1168, SA1003	*efb*[b]	gtgtttat**AGTTAA*TT***aataatt**AGTTAA*TTcaa***aagttgtataaataggataactt N_{36} ATG
SACOL2419, SA2207	*hlgA*[b]	taacgaat**AGTTAA*TT***cgaaaacgcttacaaatggattattatatatatgaactt**a** N_{27} ATG
SACOL2421, SA2208	*hlgC*[b]	taatgaac**AGTTAA*TT***ataataacgcccaaaatatattattat N_{41} ATG
SACOL2418, SA2206	*sbi*[b]	taataatt**AGTTAA*TT***tccatttgtattctcatgtgataaatt N_{36} ATG
SACOL1881, SA1638	*lukE*[c]	taatgaac**AGTTAA*TT***ttcataaataagtgaaataatctagaataaatttt**g** N_{34} ATG
SACOL1880, SA1637	*lukD*[c]	agatataa**AGTTAATT**ggaaaacacacgaaattaaagtgaaaggacataattaat ATG
SACOL1389, SA1300	*parE*	atgaa**AGTTAA*TT***gatacttgcattttacgctgatttatat N_{63} ATG
SACOL2136, SA1946	Hypothetical protein of *czr* operon	tcactaat**AGTTAA*TT***tgaaatgtggaagtttataatacgat N_{82} ATG

[a] The COS is shown in uppercase, putative −35 boxes are underlined and in italics, putative −10 boxes are underlined, and experimentally mapped transcription start points are in bold and underlined. Putative −35 and −10 boxes were identified by visual inspection of the respective promoters. The COS is also found on the complementary strand of the *abcA* (21) and *icaR* (8) promoters.

[b] SaeRS-regulated gene (39).

[c] *lukE* and *lukD* form a bicistronic operon. The COS in *lukD* is located just upstream of a ribosome binding site.

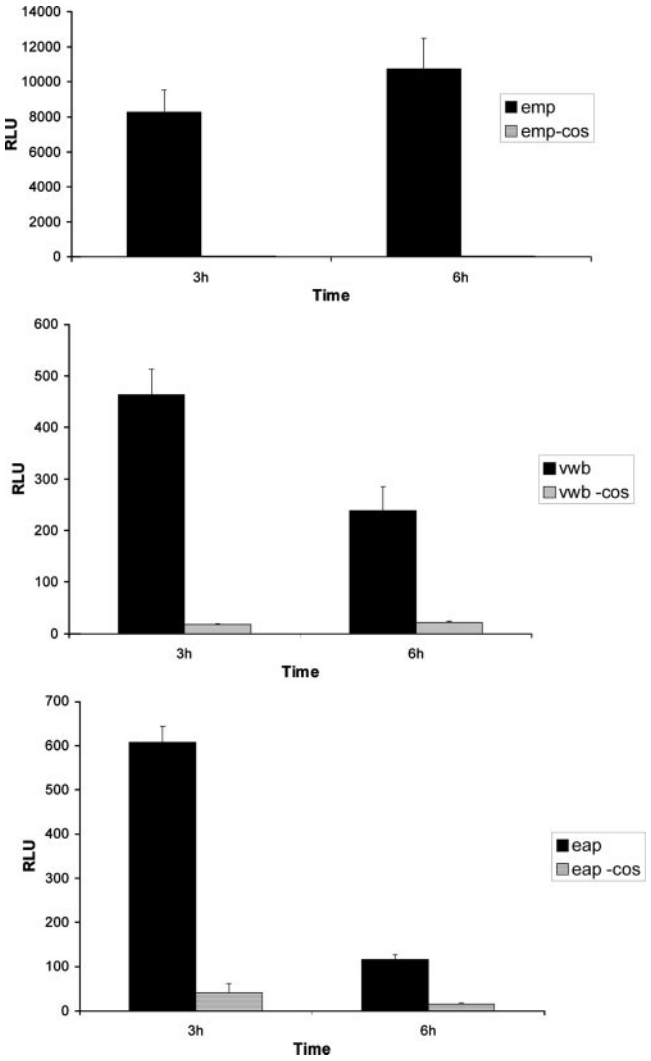

FIG. 2. Effect of deleting the COS in the *emp* (A), *vwb* (B), and *eap* (C) promoters. A β-galactosidase assay was used for measuring *emp* and *vwb* promoter activity, and the bioluminescence assay described in Harraghy et al. (19) was used for measuring *eap* expression. The data shown are the means ± standard errors of the means of the results of at least two independent experiments. RLU, relative light units.

TTCGGACTC-3′) and vwbPR1 (5′-CCTAAGCTTTAATTTT CCCTAATTAAC-3′) amplified the entire promoter region, while primers vwb-cs_F (5′-CTACCTTTTTAAAATGTTGAT GAA-3′) and vwb-cs_R (5′-ATTCATCAACATTTTAAAAA GGTAG-3′) were the complementary internal primers lacking the COS. The COS in the *eap* promoter was deleted by using a QuikChange mutagenesis kit (Stratagene) using primers QCF1 (5′-GATAATTTATTATTAATATTCCAAAAAATA GAGAAAGTCTGGC-3′) and QCR1 (5′-GCCAGACTTTCT CTATTTTTTGGAATATTAATAATAAATTATC-3′). All clones were sequenced to confirm the deletion of the COS and that no additional mutations had been introduced during cloning. The mutated promoters were cloned in their respective reporter gene vectors and transduced into strain Newman as described in reference 19. The expression of *eap* was analyzed by using a bioluminescence assay, while *emp* and *vwb* were analyzed by using a β-galactosidase assay. As shown in Fig. 2, deleting the COS in all three promoters severely repressed the expression of the reporter gene. To exclude the possibility that the deletion of the COS per se was responsible for the decrease in expression, we mutated the COS in the *emp* promoter, changing the sequence from AGTTAATT to TCATAATT (thereby changing the first three nucleotides of the COS while leaving the putative −35 box intact) by using a QuikChange mutagenesis kit (Stratagene) with primers QCF3_emp (5′-GA CAACGTTTACTTCATCATAATTATTATACTGAAAATT CTGG-3′) and QCF3_emp-r (5′-CCAGAATTTTCAGTATA ATAATTATGATGAAGTAAACGTTGTC-3′). As shown in Fig. 3, mutagenesis of the COS in the *emp* promoter resulted in a >50% decrease in *emp* expression but did not completely abrogate expression. This is likely due to the partial homology of the region to the COS. Taken together, our findings suggested that the COS could be the binding site for a regulator of *eap*, *emp*, and *vwb*.

In our previous study, we showed that *sarA* and RNAIII are involved in the regulation of *eap* and *emp* and that *sae* is essential for the expression of both genes (19). Six of the 11 genes in Table 1 are also regulated by *sae* (39). To investigate whether SaeR was binding to the *eap*, *emp*, and *vwb* promoters, SaeR was amplified from *S. aureus* Newman using primers saeR_F2 (5′-GGCATACATATGACCCACTTACTGATC-3′) and saeR_R3 (5′-CCCCCAAGCTTATCGGCTCCTTTCAA ATTTATATCC-3′), cloned in the pET28a vector (Novagen), and overexpressed in *Escherichia coli*. The purified protein was subsequently assessed for binding to each promoter (see the supplemental material for the DNA sequences used) by using electrophoretic mobility shift assays (EMSA). However, no binding of SaeR to the promoters was found (data not shown). As it is possible that SaeR needs to be phosphorylated to bind to its target promoters (13), we decided to purify the DNA binding domain of SaeR and looked for binding of this to the promoters using EMSA. However, we did not observe any binding of the SaeR DNA binding domain to the three promoters. These findings, as well as the observations that *vwb* is not regulated by *sae* (39) and that some *sae*-regulated genes, e.g., *scn* and *chp* (40), do not have a COS in their promoter, suggest that the COS is not the binding site for SaeR.

The COS is similar to a proposed binding site for SarA (AGTTAAG) (38). As SarA is known to be involved in the regulation of *eap* and *emp*, and SarA binding to different promoters has been demonstrated (6, 38), we investigated whether the COS could be a binding site for SarA. Although SarA binds to the *eap*, *emp*, and *vwb* promoters (N. Harraghy and J. Kormanec, unpublished data), deleting the COS did not have any effect on SarA binding to the three promoters (data not shown), indicating that the COS is not essential for SarA binding.

In summary, we have identified a COS in the *eap*, *emp*, and *vwb* promoters, as well as in the promoters of several genes recognized as being involved in modulation of the immune response to staphylococcal infection. The nature of the relationship between the SERAMs (5) and leukocidins is intriguing as it was recently shown that, in some strains, the expression of the Panton-Valentine leukocidin interferes with the regulation of the other major group of staphylococcal adhesins, the MSCRAMMs (27). Although it is unlikely that the leuko-

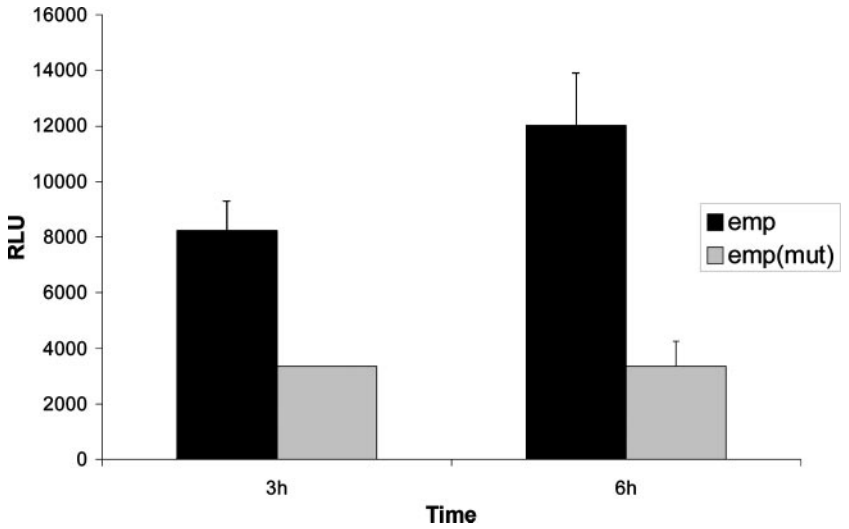

FIG. 3. Effect of mutating the COS in the *emp* promoter [emp(mut)]. The COS was changed from AGTTAATT to TCATAATT, and the effect on *emp* expression was assayed by using a β-galactosidase assay as described in reference 19. The data shown are the means ± standard errors of the means of the results of two independent experiments. RLU, relative light units.

cidins described here affect the regulation of the SERAMs, it is possible that they share a common regulator.

Our findings suggest that the COS has an important functional role because deletion of the COS in the *eap*, *emp*, and *vwb* promoters, as well as mutation of the COS in the *emp* promoter, affected the expression of the reporter gene. Although deleting the COS only partially disrupted the proposed −35 box (in the case of the *eap* promoter, there is only one mismatch in comparison with the original promoter) the deletion dramatically affected promoter activity. Moreover, the mutation of the COS in the *emp* promoter, which preserved the −35 box and maintained homology to the COS (only three bases were different), also affected promoter activity, although not to the same extent as when the COS was deleted. Thus, the changes in the expression of the reporter genes appear to be the result of modifications to the COS and the possible loss of a transcription factor-binding site. Our findings suggest that the COS is the binding site for an as-yet-unidentified regulator of *eap*, *emp*, and *vwb* that may function together with SaeR. The existence of such a factor was postulated by Goerke et al. (14) and is supported by work in our laboratory, as well as the recent findings of Kuroda et al. (26). Emerging data from microarray studies and ongoing work in our laboratory will help reveal such candidates.

We thank Sylvain Kerdudou and Markus Bischoff for critical reading of the manuscript and Karin Hilgert for excellent technical assistance.

The work in our laboratories is funded by grants from the University of Saarland HOMFOR to N.H., Deutsche Forschungsgemeinschaft grant He 1850/8-1 to M.H., and VEGA grant 2/6010/26 from the Slovak Academy of Sciences to J.K.

REFERENCES

1. **Athanasopoulos, A. N., M. Economopoulou, V. V. Orlova, A. Sobke, D. Schneider, H. Weber, H. G. Augustin, S. A. Eming, U. Schubert, T. Linn, P. P. Nawroth, M. Hussain, H. P. Hammes, M. Herrmann, K. T. Preissner, and T. Chavakis.** 2006. The extracellular adherence protein (Eap) of *Staphylococcus aureus* inhibits wound healing by interfering with host defense and repair mechanisms. Blood **107:**2720–2727.
2. **Bjerketorp, J., K. Jacobsson, and L. Frykberg.** 2004. The von Willebrand factor-binding protein (vWbp) of *Staphylococcus aureus* is a coagulase. FEMS Microbiol. Lett. **234:**309–314.
3. **Bjerketorp, J., M. Nilsson, A. Ljungh, J. I. Flock, K. Jacobsson, and L. Frykberg.** 2002. A novel von Willebrand factor binding protein expressed by *Staphylococcus aureus*. Microbiology **148:**2037–2044.
4. **Chavakis, T., M. Hussain, S. M. Kanse, G. Peters, R. G. Bretzel, J. I. Flock, M. Herrmann, and K. T. Preissner.** 2002. *Staphylococcus aureus* extracellular adherence protein serves as anti-inflammatory factor by inhibiting the recruitment of host leukocytes. Nat. Med. **8:**687–693.
5. **Chavakis, T., K. Wiechmann, K. T. Preissner, and M. Herrmann.** 2005. *Staphylococcus aureus* interactions with the endothelium: the role of bacterial "secretable expanded repertoire adhesive molecules" (SERAM) in disturbing host defense systems. Thromb. Haemost. **94:**278–285.
6. **Chien, Y., A. C. Manna, S. J. Projan, and A. L. Cheung.** 1999. SarA, a global regulator of virulence determinants in *Staphylococcus aureus*, binds to a conserved motif essential for *sar*-dependent gene regulation. J. Biol. Chem. **274:**37169–37176.
7. **Clarke, S. R., and S. J. Foster.** 2006. Surface adhesins of *Staphylococcus aureus*. Adv. Microb. Physiol. **51:**187–224.
8. **Cramton, S. E., C. Gerke, N. F. Schnell, W. W. Nichols, and F. Gotz.** 1999. The intercellular adhesion (*ica*) locus is present in *Staphylococcus aureus* and is required for biofilm formation. Infect. Immun. **67:**5427–5433.
9. **Ferrero, L., B. Cameron, B. Manse, D. Lagneaux, J. Crouzet, A. Famechon, and F. Blanche.** 1994. Cloning and primary structure of *Staphylococcus aureus* DNA topoisomerase IV: a primary target of fluoroquinolones. Mol. Microbiol. **13:**641–653.
10. **Fischetti, V. A., V. Pancholi, and O. Schneewind.** 1990. Conservation of a hexapeptide sequence in the anchor region of surface proteins from gram-positive cocci. Mol. Microbiol. **4:**1603–1605.
11. **Flock, M., and J. I. Flock.** 2001. Rebinding of extracellular adherence protein Eap to *Staphylococcus aureus* can occur through a surface-bound neutral phosphatase. J. Bacteriol. **183:**3999–4003.
12. **Geisbrecht, B. V., B. Y. Hamaoka, B. Perman, A. Zemla, and D. J. Leahy.** 2005. The crystal structures of EAP domains from *Staphylococcus aureus* reveal an unexpected homology to bacterial superantigens. J. Biol. Chem. **280:**17243–17250.
13. **Goerke, C., M. G. Bayer, and C. Wolz.** 2001. Quantification of bacterial transcripts during infection using competitive reverse transcription-PCR (RT-PCR) and LightCycler RT-PCR. Clin. Diagn. Lab. Immunol. **8:**279–282.
14. **Goerke, C., U. Fluckiger, A. Steinhuber, W. Zimmerli, and C. Wolz.** 2001. Impact of the regulatory loci *agr*, *sarA* and *sae* of *Staphylococcus aureus* on the induction of alpha-toxin during device-related infection resolved by direct quantitative transcript analysis. Mol. Microbiol. **40:**1439–1447.
15. **Gomez, M. I., A. Lee, B. Reddy, A. Muir, G. Soong, A. Pitt, A. Cheung, and A. Prince.** 2004. *Staphylococcus aureus* protein A induces airway epithelial inflammatory responses by activating TNFR1. Nat. Med. **10:**842–848.
16. **Haggar, A., C. Ehrnfelt, J. Holgersson, and J. I. Flock.** 2004. The extracellular adherence protein from *Staphylococcus aureus* inhibits neutrophil binding to endothelial cells. Infect. Immun. **72:**6164–6167.
17. **Haggar, A., O. Shannon, A. Norrby-Teglund, and J. I. Flock.** 2005. Dual

effects of extracellular adherence protein from *Staphylococcus aureus* on peripheral blood mononuclear cells. J. Infect. Dis. **192:**210–217.
18. **Harraghy, N., M. Hussain, A. Haggar, T. Chavakis, B. Sinha, M. Herrmann, and J. I. Flock.** 2003. The adhesive and immunomodulating properties of the multifunctional *Staphylococcus aureus* protein Eap. Microbiology **149:**2701–2707.
19. **Harraghy, N., J. Kormanec, C. Wolz, D. Homerova, C. Goerke, K. Ohlsen, S. Qazi, P. Hill, and M. Herrmann.** 2005. *sae* is essential for expression of the staphylococcal adhesins Eap and Emp. Microbiology **151:**1789–1800.
20. **Hawley, D. K., and W. R. McClure.** 1983. Compilation and analysis of *Escherichia coli* promoter DNA sequences. Nucleic Acids Res. **11:**2237–2255.
21. **Henze, U. U., and B. Berger-Bachi.** 1995. *Staphylococcus aureus* penicillin-binding protein 4 and intrinsic β-lactam resistance. Antimicrob. Agents Chemother. **39:**2415–2422.
22. **Higgins, J., A. Loughman, K. P. van Kessel, J. A. van Strijp, and T. J. Foster.** 2006. Clumping factor A of *Staphylococcus aureus* inhibits phagocytosis by human polymorphonuclear leucocytes. FEMS Microbiol. Lett. **258:**290–296.
23. **Hussain, M., K. Becker, C. von Eiff, J. Schrenzel, E. Peters, and M. Herrmann.** 2001. Identification and characterization of a novel 38.5-kilodalton secretory protein of *Staphylococcus aureus* with extended-spectrum binding activitiy for extracellular matrix and plasma. J. Bacteriol. **183:**6778–6786.
24. **Kaneko, J., and Y. Kamio.** 2004. Bacterial two-component and hetero-heptameric pore-forming cytolytic toxins: structures, pore-forming mechanism, and organization of the genes. Biosci. Biotechnol. Biochem. **68:**981–1003.
25. **Kreikemeyer, B., D. McDevitt, and A. Podbielski.** 2002. The role of the map protein in *Staphylococcus aureus* matrix protein and eukaryotic cell adherence. Int. J. Med. Microbiol. **292:**283–295.
26. **Kuroda, H., M. Kuroda, L. Cui, and K. Hiramatsu.** 2007. Subinhibitory concentrations of beta-lactam induce haemolytic activity in *Staphylococcus aureus* through the SaeRS two-component system. FEMS Microbiol. Lett. **268:**98–105.
27. **Labandeira-Rey, M., F. Couzon, S. Boisset, E. L. Brown, M. Bes, Y. Benito, E. M. Barbu, V. Vazquez, M. Hook, J. Etienne, F. Vandenesch, and M. G. Bowden.** 2007. *Staphylococcus aureus* Panton-Valentine leukocidin causes necrotizing pneumonia. Science **315:**1130–1133.
28. **Lee, L. Y., M. Hook, D. Haviland, R. A. Wetsel, E. O. Yonter, P. Syribeys, J. Vernachio, and E. L. Brown.** 2004. Inhibition of complement activation by a secreted *Staphylococcus aureus* protein. J. Infect. Dis. **190:**571–579.
29. **Lee, L. Y., X. Liang, M. Hook, and E. L. Brown.** 2004. Identification and characterization of the C3 binding domain of the *Staphylococcus aureus* extracellular fibrinogen-binding protein (Efb). J. Biol. Chem. **279:**50710–50716.
30. **Lee, L. Y., Y. J. Miyamoto, B. W. McIntyre, M. Hook, K. W. McCrea, D. McDevitt, and E. L. Brown.** 2002. The *Staphylococcus aureus* Map protein is an immunomodulator that interferes with T cell-mediated responses. J. Clin. Investig. **110:**1461–1471.
31. **Lowy, F. D.** 1998. *Staphylococcus aureus* infections. N. Engl. J. Med. **339:**520–532.
32. **Mazmanian, S. K., G. Liu, E. R. Jensen, E. Lenoy, and O. Schneewind.** 2000. *Staphylococcus aureus* sortase mutants defective in the display of surface proteins and in the pathogenesis of animal infections. Proc. Natl. Acad. Sci. USA **97:**5510–5515.
33. **Moran, C. P., Jr., N. Lang, S. F. LeGrice, G. Lee, M. Stephens, A. L. Sonenshein, J. Pero, and R. Losick.** 1982. Nucleotide sequences that signal the initiation of transcription and translation in *Bacillus subtilis*. Mol. Gen. Genet. **186:**339–346.
34. **Ni Eidhin, D., S. Perkins, P. Francois, P. Vaudaux, M. Hook, and T. J. Foster.** 1998. Clumping factor B (ClfB), a new surface-located fibrinogen-binding adhesin of *Staphylococcus aureus*. Mol. Microbiol. **30:**245–257.
35. **Palma, M., A. Haggar, and J. I. Flock.** 1999. Adherence of *Staphylococcus aureus* is enhanced by an endogenous secreted protein with broad binding activity. J. Bacteriol. **181:**2840–2845.
36. **Pribnow, D.** 1975. Bacteriophage T7 early promoters: nucleotide sequences of two RNA polymerase binding sites. J. Mol. Biol. **99:**419–443.
37. **Pribnow, D.** 1975. Nucleotide sequence of an RNA polymerase binding site at an early T7 promoter. Proc. Natl. Acad. Sci. USA **72:**784–788.
38. **Rechtin, T. M., A. F. Gillaspy, M. A. Schumacher, R. G. Brennan, M. S. Smeltzer, and B. K. Hurlburt.** 1999. Characterization of the SarA virulence gene regulator of *Staphylococcus aureus*. Mol. Microbiol. **33:**307–316.
39. **Rogasch, K., V. Ruhmling, J. Pane-Farre, D. Hoper, C. Weinberg, S. Fuchs, M. Schmudde, B. M. Broker, C. Wolz, M. Hecker, and S. Engelmann.** 2006. Influence of the two-component system SaeRS on global gene expression in two different *Staphylococcus aureus* strains. J. Bacteriol. **188:**7742–7758.
40. **Rooijakkers, S. H., M. Ruyken, J. van Roon, K. P. van Kessel, J. A. van Strijp, and W. J. Van Wamel.** 2006. Early expression of SCIN and CHIPS drives instant immune evasion by *Staphylococcus aureus*. Cell. Microbiol. **8:**1282–1293.
41. **Rozalska, B., and T. Wadstrom.** 1992. Interaction of fibronectin and fibronectin binding protein (FnBP) of *Staphylococcus aureus* with murine phagocytes and lymphocytes. FEMS Microbiol. Immunol. **4:**305–315.
42. **Shannon, O., and J. I. Flock.** 2004. Extracellular fibrinogen binding protein, Efb, from *Staphylococcus aureus* binds to platelets and inhibits platelet aggregation. Thromb. Haemost. **91:**779–789.
43. **Sinha, B., and M. Herrmann.** 2005. Mechanism and consequences of invasion of endothelial cells by *Staphylococcus aureus*. Thromb. Haemost. **94:**266–277.
44. **Sobke, A. C., D. Selimovic, V. Orlova, M. Hassan, T. Chavakis, A. N. Athanasopoulos, U. Schubert, M. Hussain, G. Thiel, K. T. Preissner, and M. Herrmann.** 2006. The extracellular adherence protein from *Staphylococcus aureus* abrogates angiogenic responses of endothelial cells by blocking Ras activation. FASEB J. **20:**2621–2623.
45. **Ton-That, H., S. K. Mazmanian, K. F. Faull, and O. Schneewind.** 2000. Anchoring of surface proteins to the cell wall of *Staphylococcus aureus*. Sortase catalyzed in vitro transpeptidation reaction using LPXTG peptide and NH_2-Gly_3 substrates. J. Biol. Chem. **275:**9876–9881.
46. **Wolz, C., C. Goerke, R. Landmann, W. Zimmerli, and U. Fluckiger.** 2002. Transcription of clumping factor A in attached and unattached *Staphylococcus aureus* in vitro and during device-related infection. Infect. Immun. **70:**2758–2762.
47. **Xie, C., P. Alcaide, B. V. Geisbrecht, D. Schneider, M. Herrmann, K. T. Preissner, F. W. Luscinskas, and T. Chavakis.** 2006. Suppression of experimental autoimmune encephalomyelitis by extracellular adherence protein of *Staphylococcus aureus*. J. Exp. Med. **203:**985–994.
48. **Zhang, L., K. Jacobsson, J. Vasi, M. Lindberg, and L. Frykberg.** 1998. A second IgG-binding protein in *Staphylococcus aureus*. Microbiology **144:**985–991.