ELSEVIER

FEMS Microbiology Reviews 28 (2004) 183–200

FEMS MICROBIOLOGY Reviews

www.fems-microbiology.org

Regulation of virulence determinants in *Staphylococcus aureus*: complexity and applications

Stéphane Bronner, Henri Monteil, Gilles Prévost *

Institut de Bactériologie, Faculté de Médecine, Université Louis Pasteur – Hôpitaux, Universitaires de Strasbourg, 3, rue Koeberlé, F-67000 Strasbourg, France

Received 4 February 2003; received in revised form 16 May 2003; accepted 15 September 2003

First published online 24 October 2003

Abstract

The virulence of *Staphylococcus aureus* is essentially determined by cell wall associated proteins and secreted toxins that are regulated and expressed according to growth phases and/or growth conditions. Gene expression is regulated by specific and sensitive mechanisms, most of which act at the transcriptional level. Regulatory factors constitute numerous complex networks, driving specific interactions with target gene promoters. These factors are largely regulated by two-component regulatory systems, such as the *agr*, *saeRS*, *srrAB*, *arlSR* and *lytRS* systems. These systems are sensitive to environmental signals and consist of a sensor histidine kinase and a response regulator protein. DNA-binding proteins, such as SarA and the recently identified SarA homologues (SarR, Rot, SarS, SarT, SarU), also regulate virulence factor expression. These homologues might be intermediates in the regulatory networks. The multiple pathways generated by these factors allow the bacterium to adapt to environmental conditions rapidly and specifically, and to develop infection. Precise knowledge of these regulatory mechanisms and how they control virulence factor expression would open up new perspectives for antimicrobial chemotherapy using key inhibitors of these systems.

Keywords: *Staphylococcus aureus*; Virulence factors; Regulation; Expression; Two-component regulatory systems

Contents

* Corresponding author. Tel.: +33-3-90-24-37-57; fax: +33-3-88-25-11-13.
E-mail address: gilles.prevost@medecine.u-strasbg.fr (G. Prévost).

0168-6445/$22.00
doi:10.1016/j.femsre.2003.09.003

1. Introduction

Staphylococcus aureus is a bacterium most commonly isolated from both community-acquired and nosocomial infections. This bacterium often colonises hosts asymptomatically, but can also cause infections affecting any organ. Infection may occur following the contamination of an implanted device and appears to be most common in immunodeficient subjects. Over 50 years ago, investigators selected culture conditions to optimise leucocidin production [1]. Coleman showed that the amount of alpha-hemolysin produced varies depending on growth stage, due to a regulation phenomenon [2]. The large number of newly characterised genes reveals the complexity of regulatory circuits, which is consistent with the extraordinary ability of *S. aureus* to adapt to the human host. Moreover, *S. aureus* contains genes encoding toxins, cell surface proteins and resistance to antimicrobials, which may strengthen its virulence and confer resistance to all families of antibiotics.

The extra-bacterial proteins that contribute to the virulence of *S. aureus* can be divided into five major families: adhesion proteins, superantigens, pore-forming toxins, ADP-ribosylating toxins and proteases. Although they all have similar functions, these proteins are not antigenically identical (e.g. leucotoxins and alpha-toxin, or superantigens). These virulence factors are also differently distributed among strains and are not always regulated in the same way in different strains.

Virulence factor expression appears to be controlled by the concentration of autoinducing peptides and by bacterial density, pH and CO_2, and each of these signals controls different regulatory systems. The responses of some virulence factor genes to these regulatory systems have been evaluated, providing a broad view of the different elements influencing the secretion of toxins. Some investigations about toxin expression in vivo differ from what was previously observed in vitro. Due to the pressure to design new antimicrobials, obtaining an agent that causes global down-regulation of virulence factor expression is an attractive challenge. In this review, we will discuss the recent and abundant literature on the variety and the molecular functions of regulatory factors and some perspectives for therapeutic applications.

2. Two-component regulatory systems

These regulatory systems are sensitive to environmental signals (e.g. autoinducing peptide from *agr*) and consist of two proteins: a sensor histidine kinase and a response regulator. The sensor either directly binds a specific extracellular ligand or is associated with a receptor [3]. Autophosphorylation occurs when the extracellular ligand binds to the receptor, leading to the transfer of a phosphate residue from ATP to a histidine residue of the cytoplasmic domain of the sensor kinase. This phosphate residue is further transferred to an aspartate residue of the response regulator. The phosphorylation cascade ends with the binding of the response regulator to specific DNA sequences, which activates the transcription-regulating functions. The specific target genes controlled by these DNA-binding proteins encode effectors of the two-component systems (e.g. RNAIII from *agr*) and/or various proteins. The C-terminus domain of the sensor histidine kinase (Fig. 1) and the N-terminus domain of the response regulator (Fig. 2) are highly conserved amongst bacterial species and are responsible for general regulatory processes.

In *S. aureus*, the best-described two-component system is *agrAC*, but other two-component systems involved in the regulation of virulence factors have also been described (*saeRS*, *srrAB*, *arlRS*, *lytRS*). The GenBank accession numbers for these genes are given in Table 1.

2.1. Agr (accessory gene regulator)

The *agr* system is usually described to have a dual action, as it a represses the transcription of a number of cell wall-associated proteins (protein A, coagulase, fibronectin binding protein) and activates that of several exoproteins (e.g. alpha-toxin, beta-hemolysin, TSST-1, leucotoxins) during the post-exponential phase. Dunman et al. [4] identified 104 genes that are up-regulated and 34 that are down-regulated by *agr* in a cell density-dependent manner. The effector of *agr* is a RNA molecule, RNAIII, which modulates virulence factor expression both at the transcriptional and translational levels [5,6]. The effect of *agr* on protein production has been studied by northern blot analysis [6–8], competitive RT-PCR [9,10] and oligonucleotide microarrays [4]. Table 2 shows how the *agr* locus affects the production of different proteins.

2.1.1. Genetic organisation

The insertion of a transposon (Tn*551*) into the *agr* locus has a pleiotropic effect on several extracellular and cell wall-associated proteins that are involved in adher-

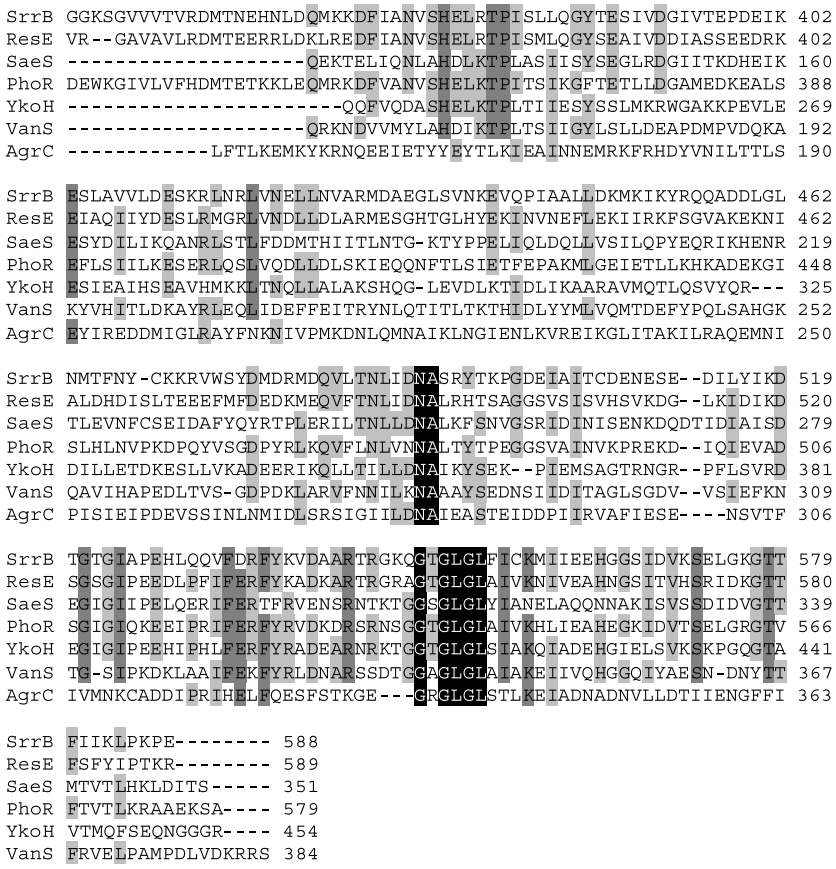

Fig. 1. Multiple sequence alignment of sensor histidine kinases SrrB, SaeS and AgrC from *S. aureus*, ResE, PhoR and YkoH from *B. subtilis*, and VanS from *E. faecium*. The alignment was made using the Clustal W (1.82) tool. Residues conserved in all seven sequences are highlighted in black, those conserved in six sequences are highlighted in dark grey and those conserved in four or five sequences are highlighted in light grey.

ence to extracellular matrix proteins essential for colonisation and infections (e.g. protein A, fibronectin-binding protein) [11].

The *agr* locus consists of five genes (*agrA*, *agrC*, *agrD*, *agrB* and *hld*), but is composed of two divergent transcripts, RNAII and RNAIII, which are under the control of two distinct promoters, P2 and P3 [12]. The P2 transcriptional unit (RNAII, 3500 nt) encodes AgrB, AgrD, AgrC and AgrA, which are required for the transcription of P2 and for the activation of P3 [5,13,14]. AgrC and AgrA function as sensor and response regulator proteins, respectively, and have similar sequences to elements of other bacterial two-component signal transduction systems [3]. The partially translated P3 transcript, RNAIII (517 nt), is the effector of the *agr* locus and also encodes the delta-hemolysin peptide (26 aa) [15], which is not involved in regulation [16].

2.1.2. Mode of action and regulation of the agr locus

In *S. aureus*, the *agr* locus is regulated by two different pathways (Fig. 3).

2.1.2.1. Autoactivation of the agr locus. The first pathway consists of the auto-activation of RNAII and RNAIII transcription by their respective P2 and P3 promoters. During the exponential growth phase, the *agrD* gene encodes a propeptide that is matured by proteolytic digestions and forms a thioester bond before being secreted into the extracellular environment. This process is carried out by the anchored membrane protein AgrB (26 kDa) containing six transmembrane segments [14,17]. The resulting mature autoinducing peptide (AIP) consists of seven to nine residues and contains a functionally critical thiolactone. It is formed by a thioester bond between the sulfhydryl group of a cysteine residue and the C-terminal carboxyl group of AIP [18,19]. In addition, the *agr* locus is activated when the AIP concentration reaches a threshold that is directly linked to specific cell density. This type of regulation, whereby the bacterial population responds to cell density, defines quorum sensing [20].

The AgrC protein (46 kDa), the transmembrane receptor (five transmembrane domains) of the AIP, contains a cytoplasmic histidine kinase domain at its C-terminus and is, thus, the sensor of the system [19]. The specific binding of the mature AIP to its receptor alters the conformation of AgrC, leading to homodimerisation and the *trans*-phosphorylation of the histidine domain

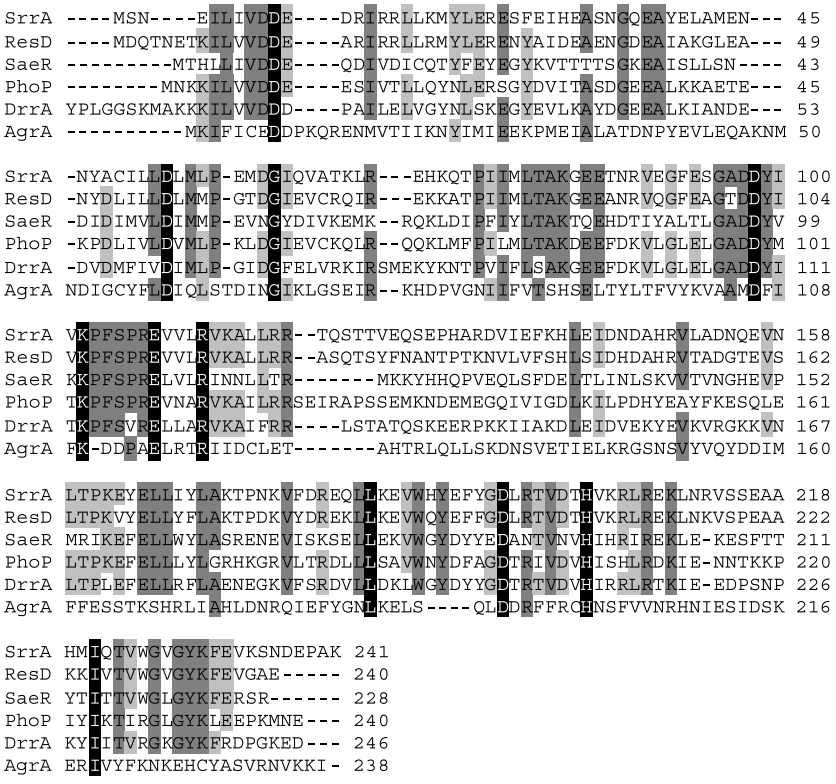

Fig. 2. Multiple sequence alignment of response regulator proteins SrrA, SaeR and AgrA from *S. aureus*, ResD and PhoP from *B. subtilis*, and DrrA from *T. maritima*. The alignment was made using the Clustal W (1.82) tool. Residues conserved in all six sequences are highlighted in black, those conserved in five sequences are highlighted in dark grey and those conserved in four sequences are highlighted in light grey.

Table 1
Accession numbers in GenBank–EMBL and TIGR databases

Gene	Organism	Accession number
agr	*S. aureus*	X52543
sae	*S. aureus*	AF129010
rap	*S. aureus*	AF205220
trap	*S. aureus*	AF202641
srr	*S. aureus*	AF260326
arl	*S. aureus*	AF165314
lyt	*S. aureus*	L42945
sarA	*S. aureus*	SAU46541
sarR	*S. aureus*	AF207707
sarS	*S. aureus*	TIGR locus name[a]:SA0096
sarT	*S. aureus*	TIGR locus name[a]:SA2506
sarU	*S. aureus*	TIGR locus name[a]:SA2507
rot	*S. aureus*	AF189239
sigB	*S. aureus*	Y09929
drrA	*T. maritima*	NP_229455
phoP	*B. subtilis*	NP_390788
phoR	*B. subtilis*	NP_390789
ykoH	*B. subtilis*	O34638
resD	*B. subtilis*	NP_390193
resE	*B. subtilis*	NP_390788
vanS	*E. faecium*	M68910

[a] TIGR genome library database: http://www.tigr.org.

[18,21]. The phosphate residue is further transferred to an aspartate residue on the cytoplasmic ArgA protein (34 kDa), leading to conformational changes of AgrA that allow it to bind the *agr* P2 and P3 promoters or to interact with other factors. The receptor/sensor AgrC and the AgrA response regulator form a two-component signal transduction pathway.

2.1.2.2. Activation of the agr locus by the RNAIII-activating protein, RAP. The RAP–TRAP two-component system, first described by Balaban and Novick [22], appears to activate the transcription of RNAII. It is hypothesised that the *agr* locus is also activated by a 38-kDa secreted protein named RAP (RNAIII-activating protein). Surprisingly, the NH_2-terminal sequence of RAP shows homology with the L2 ribosomal protein of *S. aureus*, which is encoded by the *rplB* gene (GenBank accession number AF205220) [23,24]. In fact, RAP and the ribosomal protein L2 might be the same protein with two different functions [25], but the mechanism of its secretion remains unclear. The RAP protein was purified by gel filtration from concentrated and boiled culture supernatants of *S. aureus* RN6390B (*agr*+) and RN6911 (*agr*-null) [22,26]. However, this two-component system remains controversial because these experiments have not yet been reproduced [27].

The autoinducer RAP is secreted during all the bacterial growth phases and may activate the phosphorylation of a 21-kDa protein, TRAP [(167 residues),

Table 2
Examples of *agr*-regulated genes

Protein name	Gene	Regulation	References
Capsular polysaccharide (type 5)	*cap5*	+	[107]
Capsular polysaccharide (type 8)	*cap8*	+	[108]
Protein A	*spa*	–	[109]
Fibronectin-binding protein	*fnbA*	–	[110]
	fnbB	–	[110]
Alpha-toxin	*hla*	+	[6]
Beta-hemolysin	*hlb*	+	[11]
Delta-hemolysin	*hld*	+	[11]
Gamma-hemolysin	*hlgA*	+	[9]
	hlgCB	+	[4,9]
Panton-Valentine Leucocidin	*luk-PV*	+	[9]
LukE-LukD	*lukED*	+	[9]
TSST-1	*tst*	+	[11]
Enterotoxin B	*seb*	+	[111]
Enterotoxin C	*sec*	+	[111]
Enterotoxin D	*sed*	+	[111]
Exfoliatin A and B	*eta*, *etb*	+	[112]
V8 serine protease	*sspA*	+	[113]
Proteases SlpA, B, C, D, E, F	*slpA*, *B*, *C*, *D*, *E*, *F*	+	[114]

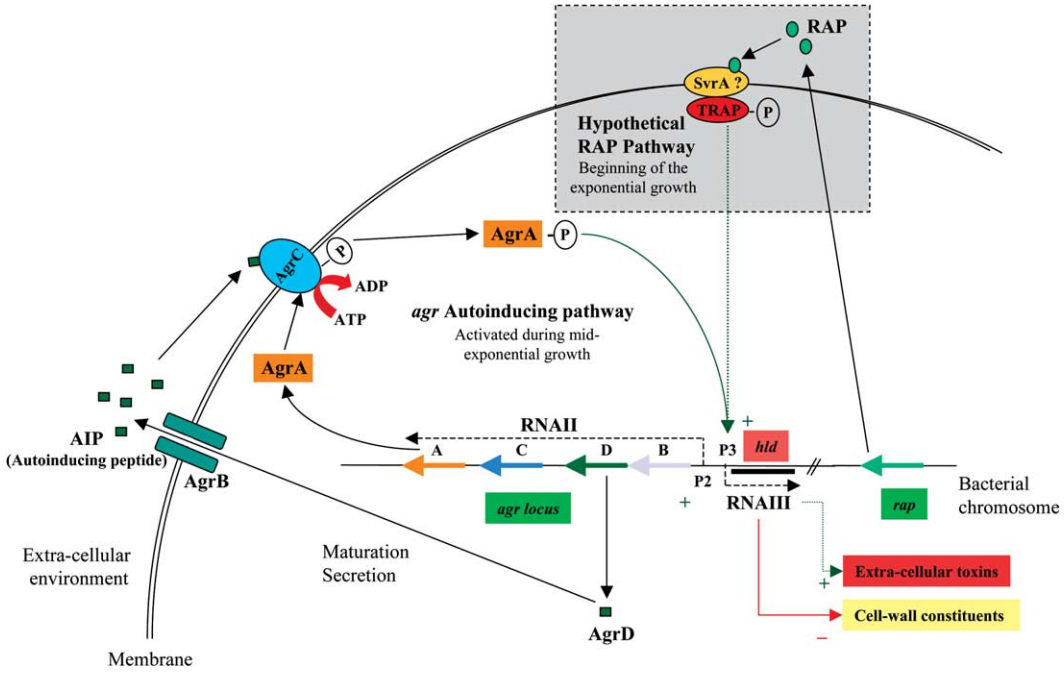

Fig. 3. Schematic figure showing how the staphylococcal two-component regulatory system, *agr*, interacts with the TRAP system (“Target of RNAIII Activating Protein”).

GenBank accession number AF202641], which then probably binds to a membrane-associated molecule [24,25]. It is believed that SvrA (staphylococcal virulence regulator) assumes this function, but this has not yet been confirmed experimentally [28]. Consequently, the phosphorylation of TRAP may activate the *agr* P2 and P3 promoters. Balaban et al. [24] suggested that the *agr* AIP produced during the mid-exponential growth phase inhibits TRAP phosphorylation, leading to the activation of a phosphatase. In this way, RAP and AIP may activate the *agr* locus in a temporal manner.

A potential inhibitor of the *agr* locus has been described. The RNAIII-inhibiting peptide (RIP) was originally isolated from coagulase-negative *Staphylococcus* species (*S. warnerii* and *S. xylosus*) [24]. The sequence of RIP is YSPXTNF, where X can be a cysteine or a tryptophan. RIP shows sequence similarity with the NH_2-terminal sequence of RAP. The RIP peptide

competes with RAP for the activation of RNAIII synthesis, probably by the binding the same receptor by an agonist-antagonist mechanism. This opens up new perspectives for therapeutic peptides.

2.1.2.3. Regulation of the RNAIII target proteins. The RNAIII molecule is the pleiotropic effector of the *agr* operon. Its secondary structure is characterised by 14 β-hairpins and three long-range interactions that bring its 3′ and 5′ ends into close proximity [29]. The main role of RNAIII is to initiate the transcription of target genes, directly or indirectly by regulating numerous transcription regulators (SarT, SarS) [30–32]. In the case of alpha-toxin (Fig. 4), RNAIII activates *hla* gene expression both at the transcriptional and at the translational levels, unlike other antisense RNAs that inhibit translation [5,6]. The binding of the 5′ end of RNAIII to an 80-bp region in the vicinity of the Shine-Dalgarno region of the *hla* mRNA results in a conformational change that allows translation to occur. These changes disrupt the intramolecular base pairing in *hla* mRNA near the ribosome binding site and thus translation. The mechanism by which RNAIII regulates gene transcription remains unknown. One hypothesis is that RNAIII forms a stable secondary structure on DNA that creates protein-binding sites [5].

2.1.3. Genetic variability of the agr locus

S. aureus strains were initially divided into three allelic groups (I, II, III) on the basis of AIP cross-inhibition of the *agr* response [19]. This means that a specific AIP belonging to one *agr* group is able to inhibit the *agr* signalling pathway of any strain from another AIP group or another staphylococcal species. Jarraud et al. [33] described a fourth *agr* group (IV), which consists of epidermolysins A- and/or B-producing *S. aureus* strains. Polymorphism of the *agr* locus results in specific differences in the *agrB*-, *agrD*- and *agrC*-encoding sequences within each group [19,33]. Indeed, the sequence of the AIPs is variable and can be associated with four *agr* specific groups, but all contain a conserved cysteine residue located five amino acids before their C-terminus [34]. This might influence co-infection by different *S. aureus* strains with different antimicrobial susceptibilities; one strain could be selected and become virulent as a result of treatment that eradicated a second susceptible strain. There is no proof that there is a direct relationship between an *agr* group and a specific human disease caused by *S. aureus* [35], but some groups seem to preferentially produce specific toxins, e.g. most menstrual toxic shock strains belonged to *agr* group III [19] and most epidermolysin-A-producing strains belong to *agr* group IV [33].

2.2. sae (S. aureus exoprotein expression)

The *sae* locus was first described by Giraudo et al. [36], following the characterisation of a Tn*551* insertional mutant of *S. aureus* strain RC106. This mutant exhibited altered virulence factor production in broth culture supernatant (α- and β-hemolysins, DNase, coagulase and protein A), but exhibited a distinct phenotype from other regulatory mutants. Like *agr*, the *sae*

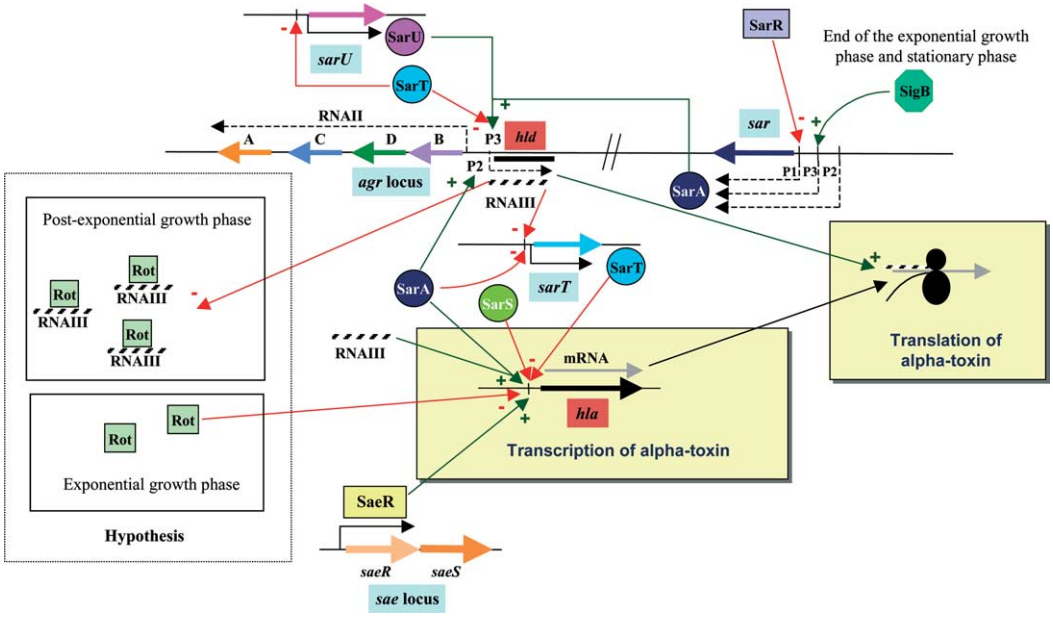

Fig. 4. Schematic figure showing how the expression of *hla* (alpha-toxin) is regulated in *S. aureus* by multiple interactions between regulatory factors. Green and red arrows indicate positive and negative regulation, respectively.

locus regulates gene expression primarily at the transcriptional level [37].

2.2.1. Genetic organisation

The *sae* locus is a two-component regulatory system constituted by two co-transcribed genes, *saeR* (687 bp) and *saeS* (1062 bp), separated by two nucleotides [38]. The SaeR protein (26.9 kDa) shows a high level of sequence identity with other regulatory proteins, such as DrrA from *Thermotoga maritima* or ResD and PhoP from *Bacillus subtilis* (Fig. 2). The N-terminal region of SaeR contains an aspartate phosphorylation site that is highly conserved among response regulators [39]. The conserved C-terminus of SaeS (39.5 kDa) has an auto-phosphorylated histidine residue and shows a high level of sequence identity with sensor histidine protein kinases, such as PhoR and YkoH from *B. subtilis* and VanS from *Enterococcus faecium* [38] (Fig. 1). The N-terminal region of the SaeS protein contains two transmembrane domains that are also found in other sensor proteins [39]. Nevertheless, there are low levels of identity between SaeS–SaeR proteins and AgrC–AgrA proteins [38] (Figs. 1 and 2).

2.2.2. Regulatory properties

The SaeS–SaeR system can be considered to be a major regulatory system as the *sae* locus is essential for the transcription of *hla*, *hlb* and *coa* but does not affect the expression of *agr* or *sarA* [37]. Indeed, *sae* activity might be independent from *agr* and *sarA*. Light-cycling reverse transcription-polymerase chain reaction (PCR) showed that the level of *hla* transcripts is considerably decreased in *sae* mutated strains, both in vitro and in vivo [40]. The same investigators observed the *sae*-dependent and *agr*/*sarA*-independent activation of *hla* in exudates accumulated in a guinea pig model with an implanted device-originated infection. *sae* may be an additional regulator of virulence factors in vivo.

2.3. srrAB (Staphylococcal respiratory response) or srhSR

S. aureus is a facultative anaerobe, able to grow in the absence of oxygen either by anaerobic respiration with nitrate as the terminal electron acceptor or by carbohydrate fermentation. At the site of infection, oxygen rapidly becomes a limiting factor, especially in deep infections. However, the expression of some virulence factors, e.g. alpha-toxin, requires oxygen [41].

The *srrAB* (or *srhSR*) system was simultaneously described by two different groups as being involved in regulation influenced by environmental oxygen conditions [42,43].

2.3.1. Genetic organisation

The *srrAB* locus is composed of two open reading frames that overlap over 20 nucleotides. The two genes, *srrA* (762 bp) and *srrB* (1752 bp), are co-transcribed, but *srrA* can be transcribed independently of *srrB*.

2.3.2. Regulatory properties

SrrA (SrhS) and SrrB (SrhR) have similar sequences to ResDE, the two-component respiratory and anaerobic fermentation regulation system from *B. subtilis* [44,45]. The sequences of the 28-kDa response regulator protein, SrrA, and that of the 66-kDa histidine kinase, SrrB, display a high level of identity with ResD and ResE, respectively (Figs. 1 and 2). SrrB contains a conserved histidine residue and two predicted transmembrane domains in its N-terminus, like most two-component membrane sensor histidine kinases [3].

Yarwood et al. [43] proposed that SrrAB may provide a link between respiratory metabolism, an environmental signal (oxygen level) and the regulation of virulence factors in *S. aureus*.

Indeed, SrrAB (SrhSR) regulates a number of proteins involved in energy metabolism and is required for the fermentative growth of *S. aureus*, e.g. by up-regulating the enzymes involved in fermentation (alcohol dehydrogenase, L-lactate dehydrogenase) in anaerobic conditions [42]. Moreover, *srrAB* is overexpressed in anaerobic conditions. Consequently, the expression of some virulence genes (e.g. TSST-1) may be down-regulated by SrrAB, in part independently of the RNAIII level and in part by the repression of RNAIII transcription [43]. This may explain menstrual toxic shock syndrome (TSS), where TSST-1 may be stimulated by an increased level of oxygen due to the insertion of a tampon into the normally anaerobic vaginal environment [46].

2.4. ArlSR (Autolysis-related locus)

The role of autolysins in the growth process is to split the wall to allow cell division and separation. They are also involved in peptidoglycan turnover and in the enlargement of the peptidoglycan sacculus. Consequently, the autolysins must be carefully controlled; indeed, they are also involved in cell suicide. Fournier et al. [47] described the *arlSR* locus after studying the production of *S. aureus* NorA, a multidrug transporter, in the presence of a transposon. The transposon, located near the 5′ terminus of the *arlS* gene, resulted in increased *norA* expression.

2.4.1. Genetic organisation

The *arlS–arlR* locus is composed of two overlapping open reading frames, *arlS* and *arlR*. These two genes are co-transcribed resulting in a 2700-bp mRNA.

2.4.2. Regulatory properties

The *arl* locus is transcribed in a growth phase-dependent manner, from the exponential to the

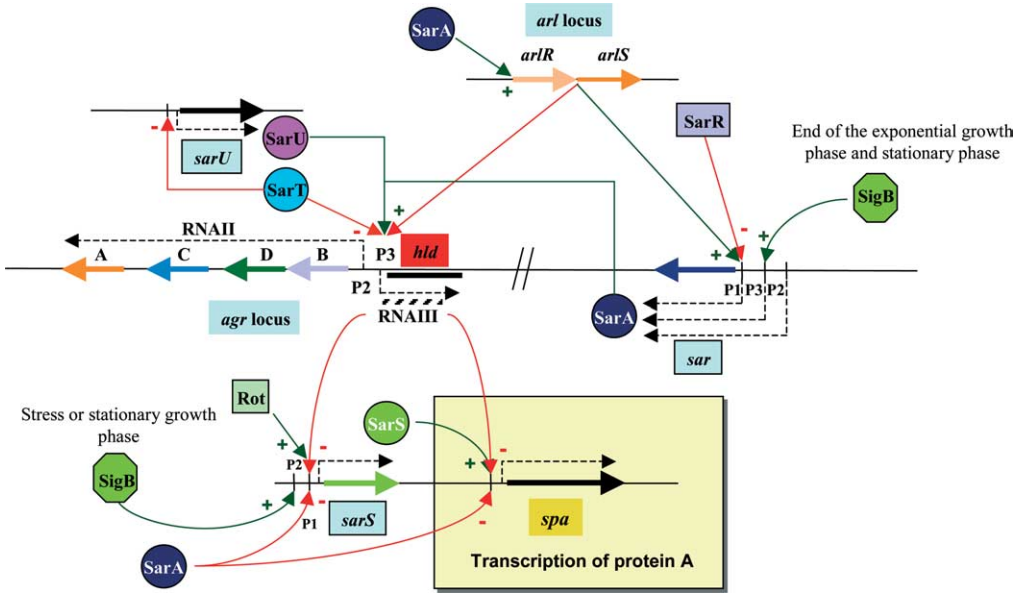

Fig. 5. Schematic figure showing how the expression of *spa* (Protein A) is regulated in *S. aureus* by multiple interactions between regulators. Green and red arrows indicate positive and negative regulation, respectively.

post-exponential growth phase. ArlS (52.4 kDa) is the sensor protein of this two-component system. It exhibits strong similarities with histidine kinase proteins and contains a conserved histidine residue, but the activating environmental signal remains unknown. ArlR (25.5 kDa) is the response regulator protein and belongs to the PhoB-OmpR family. It contains a conserved C-terminal domain that binds to specific DNA sequences located upstream of the target promoters. The disruption of the *arlS* gene increases peptidoglycan hydrolase activity, leading to its autolysis. Therefore, ArlS and ArlR might be involved in cell growth and division, and in the control of attachment to polymer surfaces [47,48]. The *arl* system is indirectly involved in the down-regulation of the transcription of some virulence genes (α-toxin, β-hemolysin, lipase, serine protease, coagulase and protein A). Indeed, protein A and a serine protease are down-regulated as a result of the previous activation of the expression of the *sarA* locus by ArlS–ArlR (Fig. 5). The targets of ArlR may be the *sarA* regulating factors, SarR, SigmaB and SarA [49]. The *arl* system also affects the level of the *agr* AIP by down-regulating RNAII transcription, consequently decreasing the activation of the *agr* locus and the expression of the virulence genes regulated by RNAIII. ArlSR might down-regulate the transcription of *agr*-targetted genes during particular physiological phases (division, cell growth) at the site of infection.

2.5. LytRS

Like Arl, the LytR–LytS two-component system is also involved in autolysis [50]. Compared to wild-type, *lytS* mutants exhibit increased autolysis and an altered level of murein hydrolase activity.

2.5.1. Genetic organisation

The *lytS* (nt 92–1843) and *lytR* (nt 1849–2585) genes (GenBank accession number L42945) are separated by 5 nt [50]. The genes are co-transcribed, resulting in a 2500-bp mRNA.

2.5.2. Regulatory properties

The sequences of the *lytS* and *lytR* genes are similar to those of other two-component sensor histidine kinase and regulatory proteins, respectively. However, unlike most sensor proteins (two transmembrane domains), the LytS protein is predicted to contain six transmembrane domains at its N-terminus [51]. This structure, consisting of alternating hydrophobic and hydrophilic domains, is also similar to that of transport proteins, suggesting that the transduction of environmental stimuli by LytS involves transport.

LytR and LytS positively regulate the expression of two genes, *lrgA* and *lrgB*, located immediately downstream of *lytS* and *lytR*. LrgA (16.3 kDa), encoded by the *lrgA* gene, shares common and characteristic sequences with the bacteriophage-encoded holin proteins involved in murein hydrolase transport [51]. LrgA and LrgB inhibit the extracellular activity of murein hydrolases. These enzymes catalyse the cleavage of specific structural components of the bacterial cell wall, particularly during the blockade of the enlargement process by beta-lactams. The cell wall is not able to divide and the bacteria are unable to grow if these autolysins are inhibited. The strains become thus penicillin-tolerant [52].

3. The staphylococcal accessory regulator, sarA, and the sarA-homologues, sarR, T, S...

3.1. The sarA locus

The *sarA* operon was initially described by Cheung et al. [53] after screening a Tn*917* insertion library for fibrinogen-binding protein-deficient mutants. The insertion of the Tn*917* LTV1 transposon into a region of the *S. aureus* chromosome distinct from the *agr* locus, resulted in a pleiotropic effect on the expression of a number of extracellular and cell wall-associated virulence proteins.

3.1.1. Genetic organisation

The *sarA* operon was cloned and sequenced by Cheung and Projan [54]. The *sarA* operon consists of three overlapping transcripts, with common 3′ ends. The transcripts, *sarP1* (0.58 kb), *sarP3* (0.84 kb) and *sarP2* (1.15 kb), previously termed *sarA*, *sarC*, and *sarB*, respectively, are controlled by the three distinct promoters (P1, P3 and P2, respectively) [55,56]. The three transcripts encode the 14.5-kDa SarA protein [55].

3.1.2. Regulation of the sarA operon

The expression of *sarA* is growth phase-dependent. The P3 promoter has a similar sequence to stress response sigma B-dependent promoters, with a typical −10 promoter box (GGGTAT) [55,57,58]. The transcription of *sarP1* and *sarP2* is initiated by the σ^A-specific promoters P1 and P2, respectively. The multi-σ-dependent promoters of the *sarA* operon allow the expression of *sarA* during all growth phases; *sarP1* and sar*P2* from mid to exponential growth phase, and *sarP3* during the late-exponential and stationary phase or during environmental stress [58]. SarA activates its own expression and is down-regulated by SarR. The influence of the SigB factor on the expression of *sarA* remains unclear and discrepant results have been reported. These differences may be due to the strains or the environmental and growth conditions used in these studies [58–60]. Moreover, the determination of the exact role of the σ^B factor in *S. aureus* has been impeded by the presence of the natural *rsbU* deletion in two strains (derived from 8325, i.e. RN6390, 8325-4) [58,60]. However, Cheung et al. [61] detected no *sar* P3 promoter activity in vitro or in vivo in the RN6390 strain.

Due to the fact that it is regulated by multiple factors, the expression of *sarA* peaks during the late exponential phase [56]. However, the SarA protein might be regulated at the post-translational level because its level remains constant, even though the level of the corresponding mRNA varies slightly [62,63].

3.1.3. Mode of action

The SarA protein is a DNA-binding protein, which binds as a homodimer structure to a conserved A/T-rich recognition motif in the promoter regions of target genes [64]. The SarA monomer (124 residues) contains four α-helices, a short β-hairpin and a long C-terminal loop (α1α2α3–β1β2–α4-C loop) [63]. SarA was initially described as an activator of the *agr* operon; it binds to the *agr* P2 and P3 promoter regions, thus increasing the levels of both RNAII and RNAIII, and altering the synthesis of virulence factors (*agr*-dependent pathway) [65,66]. SarA can also directly bind to conserved regions, termed Sar boxes, within the promoters of several cell wall-associated proteins (protein A, fibronectin-binding proteins, collagen adhesin) and exoproteins (α-toxin, δ-hemolysin, γ-hemolysin) (*agr*-independent pathway) [4,64]. DNaseI footprinting experiments have revealed three SarA-binding sites upstream of the P2 and P3 *agr* promoters, each consisting of two half-sites [67]. The binding of the SarA dimer to the DNA-binding site alters its superhelicity, hence decreasing the number of base pairs per turn. Thus, the SarA modified the spacing between the −10 and −35 regions of promoters, leading either to optimal spacing for the transcription and up-regulation of promoters (e.g. *agr*-P2 and -P3) or to incorrect spacing for the repressed promoters (e.g. *cna* gene) [67]. The pleiotropic activity of SarA on gene expression is summarised in Table 3.

Table 3
Example of *sarA*-regulated genes

Name	Gene	Regulation	References
Capsular polysaccharide (type 8)	*cap8*	+	[108]
Protein A	*spa*	−	[4]
Fibronectin-binding protein	*fnbA*	+	[110]
	fnbB	+	[110]
Collagen Adhesin	*cna*	−	[62]
AgrA, B, C, D	*agr A, B, C, D*	+	[65]
RNAIII		+	[65]
Alpha-toxin	*hla*	+	[60]
Delta-hemolysin	*hld*	+	[4]
Gamma-hemolysin	*hlgA*	+	[9]
	hlgCB	+	[4,9]
Panton-Valentine Leucocidin	*lpv*	+	[9]
LukE-LukD	*lukED*	+	[9]
TSST-1	*tst*	+	[113]
Enterotoxin B	*seb*	+	[113]
V8 serine protease	*sspA*	−	[113]

3.2. The sarA homologues

Sequences that share a high level of identity with SarA have been identified in the *S. aureus* genome (The Institute for Genome Research, TIGR) and designated *sarA* homologues (Fig. 6). These factors belong to the winged-helix family of regulatory DNA-binding proteins, and have a basic p*I* and a high percentage of charged residues. The winged-helix proteins belong to the helix–turn–helix family of proteins, and consist of

```
SarA  ----------------------MAITKINDCFELLSMVTYADKLKSLIKKEFSISFEEF 37
SarT  ---------------------MMNDLKSKSNIKLMKRVLTTYELRKYLKKYFCLTLDNY 38
SarS1 ----------------------MKYNNHDKIRDFIIIEAYMFRFKKKVKPEVDMTIKEF 37
SarU1 ----------------------MDYQTFEKVNKFINVEAYIFFLTQELKQQYKLSLKEL 37
SarS2 -----------------LADQSESQMIPKDSKEFLNLMMYTMYFKNIIKKHLTLSFVEF 42
SarU2 ----------KIIKGFNKERDYIKYQWAPKYSKEFFILFMNIMYSKDFLKYRFNLTFLDL 50
SarR  -------------------------MSKINDINDLVNATFQVKKFFRDTKKKFNLNYEEI 35
Rot   MFVNTCMVAKYVIINWEMFSMKKVNNDTVFGILQLETLLGDINSIFSEIESEYKMSREEI 60

SarA  AVLTYISENKEKE--YYLKDIINHLNYKQPQVVKAVKILSQEDYFDKKRNEHDERTVLIL 95
SarT  LVLAYLDVFKNDEGKYFMRDIISYIGIDQSRIVKSVKELSKKGYLNKQRDPHDSRNVIIV 98
SarS1 IILTYLFHQQENT--LPFKKIVSDLCYKQSDLVQHIKVLVKHSYISKVRSKIDERNTYIS 95
SarU1 LILAYFYYKNEHS--ISLKEIIGDILYKQSDVVKNIKSLSKKGFINKSRNEADERRIFVS 95
SarS2 TILAIITSQNKNI--VLLKDLIETIHHKYPQTVRALNNLKKQGYLIKERSTEDERKILIH 100
SarU2 SILYVISSRKNEI--LNLKDLFESIRFMYPQIVRSVNRLNNKGMLIKERSLADERIVLIK 108
SarR  YILNHILRSESNE--ISSKEIAKCSEFKPYYLTKALQKLKDLKLLSKKRSLQDERTVIVY 93
Rot   LILLTLWQKGSMT----LKEMDRFVEVKPYKRTRTYNNLVELEWIYKERPVDDERTVIIH 116

SarA  VNAQQRKKIESLLSRVNKRITEANNEIEL-------- 124
SarT  VSVKQHNYIKNLLSEININET---------------- 119
SarS1 ISEEQREKIAERVTLFDQIIKQFN------------- 119
SarU1 VTPIQRKKIACVINELD-------------------- 112
SarS2 MDDAQQDHAEQLLAQVNQLLADKDHLHLVFE------ 131
SarU2 INKIQYNTIKSIFTDTSKILKPRKFFF---------- 135
SarR  VTDTQKANIQKLISELEEYIKN--------------- 115
Rot   FNEKLQQEKVELLNFISDAIASRATAMQNSLNAIIAV 153
```

Fig. 6. Multiple sequence alignment of SarA homologues from *S. aureus*. SarS and SarU are separated into two half domains, SarS1/SarS2 and SarU1/SarU2, respectively. The residues highlighted in black are conserved in all sequences, those in dark grey are identical in seven sequences and those in light grey are identical in four, five, or six of the sequences. Sequences were aligned by the Clustal W (1.82) tool.

three α-helices, three β-strands and two loops (or wings) [68].

3.2.1. SarR

SarR is a protein involved in the regulation of *sarA* expression. Manna et al. [58] partially purified a 12-kDa protein that binds to the P2 promoter region of *sarA* and to a 34-bp sequence upstream of P1, and that was predicted to repress *sarA* expression. The SarR protein (13.6 kDa) is encoded by a 345-bp gene, *sarR* [56], and has the characteristic properties of a DNA-binding protein: a basic p*I*, a high percentage of charged amino acids and a helix–turn–helix motif. Furthermore, some regions of SarR share a high level of homology with specific regions of SarA. However, the structure of SarR differs from that of SarA; it contains five α-helices, three short β-strands and several loops (α1α2–β1α3α4–β2β3α5), but can also form dimers like SarA [69,70]. The expression of *sarR* peaks during the post-exponential growth phase, leading to a decrease in *sarA* transcription during the late exponential and stationary growth phases [56].

3.2.2. SarS (SarH1)

The 29-kDa SarH1 protein was isolated by Tegmark et al. [31], because of its ability to bind the DNA promoter regions of various genes such as *hld*, *ssp*, *hla* and *spa*. Concurrently, the same protein was identified by Cheung et al. [71] and was named SarS. The *sarS* gene is located on the chromosome between the staphylococcal iron-regulated genes A, B, C (*sirABC*) and the protein A gene (*spaA*). Tegmark et al. [31] carried out Northern blotting analysis, showing that the transcription of *sarS* generates three transcripts (approximately 1000, 1500 and 3000 nt). Conversely, the more stringent northern blot conditions used by Cheung et al. [71] detected only one 930-nt transcript. Furthermore, *sarS* is transcribed from two distinct promoters: an σ^A-dependent promoter and the σ^B-dependent stress response promoter. In the study by Cheung et al. [71], the unique 930-nt transcript detected was probably the consequence of the mainly σ^A-dependent promoter activation, due to culture conditions.

The SarS protein contains two SarA-related domains (SarS1, SarS2), each containing 125 residues. They share a high degree of identity with SarA: 34.5% and 28.3% for the C-terminal and the N-terminal domains, respectively (Fig. 6). The expression of *sarS* is repressed by both *sarA* and *agr*. SarS binds the *spa* promoter region and activates its transcription. In this way, SarS may act as an intermediate in the regulation of *spa* expression controlled by both *agr* and *sarA* (Fig. 5). The *agr* locus down-regulates the expression of *spa* by a *sarS*-dependent pathway, whereas SarA down-regulates *spa* transcription in a *sarS*-independent pathway. Furthermore, the expression of the alpha-toxin gene is strongly repressed by SarS [31].

3.2.3. SarT

The 16.1-kDa SarT protein has DNA-binding properties, a high percentage of charged residues (43%) and a p*I* of 9.55 [32]. The SarT sequence is 35% identical with

that of SarA and 20% identical with that of SarR (Fig. 6).

SarT represses the expression of the alpha-hemolysin gene, but *sarT* is down-regulated either by *sarA* or *agr* [32]. The expression of *hla* is higher in a *sarA sarT* mutant than in a *sarA* mutant, suggesting that *sarA* activates *hla* transcription either by interacting with the *hla* promoter or by repressing *sarT*. The level of *hla* expression is similar in a *agr sarT* double mutant and in a *agr* mutant, indicating that *agr* does not repress *sarT* to activate *hla*. The amount of RNAIII mRNA increases significantly in *sarT* mutants, indicating that SarT might repress RNAIII transcription. Thus, SarT may repress *hla* expression, partly by down-regulating RNAIII expression. A negative feedback loop might thus exist, constituted by SarT, RNAIII and another SarA homologue, SarU.

3.2.4. SarU

SarU, a 247-residue protein with a molecular mass of 29.3 kDa, was recently described by Manna and Cheung [72]. Like SarS, the SarU protein is formed by two half domains, each sharing sequence similarities with SarA, SarR and SarT (Fig. 6). The transcription of *sarU*, initiated by a σ^A-promoter, is higher in a *sarT* mutant than in the parental strain. Moreover, a *sarU* mutation decreases the expression of RNAII and RNAIII from the *agr* locus and the expression of *agr*-target genes (e.g. *hla*) compared to in the parental strain, but increases the expression of genes encoding cell wall-associated proteins (i.e. *coa*) [72]. SarU may be involved in an *agr*-regulatory pathway that includes SarT. Indeed, *sarU* transcription is repressed by SarT, which decreases the level of RNAIII mRNA. RNAIII mRNA is also able to repress the expression of *sarT*. This constitutes a feedback loop that regulates the expression of *agr* RNAIII and virulence factors. This mechanism may be an alternative pathway for the regulation of the RNAIII expression in vivo, where bacterial densities are low and the *agr* AIP threshold is not reached in the quorum-sensing way. However, the expression level of the protein A gene (*spa*) is not higher in a *sarU* defective strain than in the parental strain in spite of the lower RNAII and RNAIII levels. Thus, *sarU* regulates various target genes via an *agr*-independent pathway that may involve other factors.

3.2.5. Rot "repressor of toxin"

Rot (15.6 kDa) was first classified as a transcription repressor. It was identified by McNamara et al. [30]. In these experiments, the transposon-mediated inactivation of the *rot* gene in an *agr*-null *S. aureus* strain partially restored protease and alpha-toxin activities. This SarA homologue shares limited sequence identity with AgrA and SarA (Fig. 6), and exhibits an acid p*I* (4.8). Recently, GeneChip analysis done by Saïd-Salim et al. [73] revealed 60 genes that are negatively regulated by Rot and 86 genes that are positively regulated by Rot. The term "repressor of protein" is thus erroneous. Moreover, Rot seems to have opposite effects on gene expression to *agr*. For example, the protein A gene (*spa*) and the serine protease genes (*sspB*, *sspC*) are up-regulated by Rot and down-regulated by *agr*, whereas the alpha-toxin gene (*hla*) and the gamma-hemolysin gene (*hlgB* and *hlgC*) are down-regulated by Rot and up-regulated by *agr* [73]. Rot might be inhibited by the RNAIII post-transcriptionaly, but the mechanism involved remains unknown. McNamara et al. [30] showed that Rot interacts with the promoter regions of target genes during the exponential growth phase (e.g. *hla*) and with RNAIII during the post-exponential growth phase. The *agr* RNAIII mRNA might repress *rot* expression. Indeed, the expression of *hla* is higher in a *rot* single mutant strain than in a *rot agr* double mutant strain. Rot may favour colonisation during the infection, because of its ability to up-regulate the expression of genes involved in adhesion, and help the bacterium to evade the host's immune response. It may also be able to down-regulate toxins or proteases involved in the later steps of infection.

3.2.6. Other sarA homologues

The *S. aureus* genome (MW2, N315) also contains some other putative *sarA* homologues. One additional factor is similar to SarR and SarT and has a basic p*I*. It was called SarY (29.8 kDa, 247 residues). SarV (13.9 kDa, 116 residues) and SarX (16.7 kDa, 141 residues) were more similar to Rot [74] (The Institute for Genomic Research – The Comprehensive Microbial Resource TIGR: http://www.tigr.org/).

4. The sigma factor σ^B

In some bacteria, the RNA polymerase (RNAP) core enzyme is composed of four subunits ($\alpha_2\beta\beta'$). The association of the core enzyme with a σ factor forms an RNAP holoenzyme that can recognise specific promoter elements, leading to the initiation of the transcription. One group of sigma factors is required for the expression of housekeeping genes and a second contains factors (σ^B) that are involved in the regulation of the gene expression upon changes in the environment (starvation, heat shock, osmotic shock, etc.). Wu et al. [75] first described and sequenced a chromosomal cluster of four *S. aureus* genes (*rsbU*, *rsbV*, *rsbW*, *sigB*), the expression products of which showed high levels of identity with those of the *sigB* operon from *B. subtilis*. These similarities suggest an analogous post-transcriptional regulation of σ^B in *S. aureus* and *B. subtilis*.

The regulation of the σ^B activity is modulated by the *rsbU*, *-V*, and *-W* gene products. RsbW acts as an

anti-sigma factor by binding σ^B and forming a RsbW–σ^B complex. RsbV is an anti-anti-σ^B factor, which competes with σ^B for RsbW. RsbV is modulated by both the phosphatase RsbU and the phosphorylase RsbW. In *B. subtilis*, σ^B is activated by two different pathways depending on the stress conditions [76,77]. The first is the RsbU-dependent pathway, which is activated when environmental stress occurs, such as ethanol, salt or heat shock. The RsbW anti-σ^B factor inactivates σ^B through the complex RsbW–σ^B, and thus suppresses the aggregation of the σ^B subunit with the RNA polymerase core enzyme [78]. During the exponential growth phase, RsbV is phosphorylated and inactivated by RsbW. Consequently, the σ^B factor is sequestered in a RsbW–σ^B complex. In response to environmental stress, RsbV is dephosphorylated by RsbU, leading to the formation of the highly specific RsbV–RsbW complex and the release of activated σ^B factor, which is able to bind to the RNAP core enzyme to form an active σ^B-holoenzyme. The second pathway, the RsbU-independent pathway, is used in conditions that reduce the intracellular level of ATP (stationary growth phase, glucose or phosphate starvation). RsbV is not phosphorylated and binds directly RsbW, which leads to the release of σ^B. Only the RsbU-dependent pathway has been demonstrated in *S. aureus*. Nevertheless, the *rsbU* natural mutants (e.g. RN6390) can still produce a functional σ^B protein, suggesting that the activity of σ^B is also dependent on factors other than RsbU. Thus, it has been suggested that a second σ^B activation pathway that is independent of RsbU but dependent on RsbV exists [79].

At least *S. aureus* 30 genes, most of which are involved in stress responses, have been shown to be controlled by σ^B [80]. Amongst them, SigB is involved in the bacterial aggregation by modulating the expression of the genes encoding clumping factor or other adhesins. This factor decreases the susceptibility of bacteria to hydrogen peroxide, possibly by affecting at least one catalase. This means that it protects against the superoxide anions released by polymorphonuclear neutrophils during the oxidative burst [76]. Moreover, σ^B might be involved in antimicrobial resistance as a *sigB S. aureus* COL mutant is considerably less resistant to methicillin than is its parental strain [81]. The protection of the bacteria against UV radiation might involve SigB, by modulating the pigmentation by carotenoids, which act as antioxidants [82].

5. Miscellaneous regulation

Based on the observation that TSST-1-producing strains generally produce low levels of other toxins, Vojtov et al. [83] proposed that in vivo both TSST-1 and enterotoxin B are global negative regulators of a series of other virulence factors, but positive regulators of their own expression. However, these analyses were done with genes located on recombinant plasmids and some strains were observed in vitro to produce several toxins including TSST-1, e.g. enterotoxin A, Panton-Valentine leucocidin, LukE-LukD leucotoxin. In addition, Yarwood et al. [84] observed that *agr* disruption or anti-SEB immunisation did not affect virulence factor expression, suggesting that this regulation only operates inside bacteria. Such observations made on recombinant genes, in a limited number of strains and with large infection inoculi might be biased.

The expression of the *pls* gene, which is located in the vicinity of *mec* and contributes to methicillin resistance and encodes a membrane associated protein may affect the expression of other staphylococcal surface proteins, thus diminishing adhesion properties [85].

6. Conclusions about the regulating factors

The regulation of the virulence gene in *S. aureus* involves a complex network of regulatory factors that interact (e.g. *hla*, Fig. 4). The publication of the complete genome sequence of *S. aureus* revealed two new groups of factors involved in regulation that share sequence similarities with the well-described factors *agr* and *sarA*. The first group contains factors that act primarily on the main regulatory systems *agr* and *sarA*, e.g. SigB and SarR, which regulate *sarA* expression. Other factors act as intermediates between the best-studied regulatory systems (*agr*, *sarA*) and the target genes (e.g. SarS, Rot, and SarT). For example, the expression of *hla* in vitro peaks at the end of the exponential growth phase. During the beginning of the growth phase, the transcription of *hla* is inhibited by Rot, SarT and SarS. During the exponential- and post-exponential phases, RNAIII transcription is increased by SarA, SarU and the two quorum sensing pathways, AIP and RAP. This blocks the inhibitors of *hla* expression, Rot, SarT and SarS, by an unknown mechanism. Consequently, *hla* transcription and translation increase. In vivo, the transcription of *hla* might be modulated by the *sae* locus [40]. Consequently, in vitro and in vivo studies in particular conditions only provide hypotheses about modes of *hla* regulation. Conversely, the expression of *spa* is upregulated at the beginning of the exponential growth phase (Fig. 5). Its transcription is up-regulated by SarS, which is itself up-regulated by the Rot factor [73]. When the amounts of RNAIII and SarA are increased during the exponential growth phase, *sarS* transcription is inhibited by these factors, meaning that *spa* transcription decreases. RNAIII and SarA also directly down-regulate *spa* expression.

During bacterial infections, various genes must be expressed in a temporally co-ordinated manner to allow

the bacterium to adapt to the environment in the host and to cause infection. Therefore, the multiplicity of these virulence regulatory factors and the interconnected networks that they form may be required to adapt to most environments.

7. Impact of regulatory systems in vivo

The first approaches used to evaluate regulatory systems in vivo involved allelic replacement and mutated strains. However, the allelic replacement approach only reaches its full potential when the considered gene modulates markers of expression directly and exclusively. Thus, some other factors remain to be considered before we can consider animal models of infection. Another critical feature is the choice of animals and the virulence factors used as markers of infection. Effectively, staphylococcal leucotoxins may not be effective in adult mice, whereas they may be effective in human cells or rabbit tissues, e.g. pore-forming toxins, epidermolysins [86]. Moreover, infections are generally initiated by quite a low number of bacteria. Therefore, the use of a large inoculum in experimental models may bias the study.

Only a few experimental models have been used to evaluate the concerted actions of *agr* and *sarA* in regulating virulence factors. These are models of mastitis, endophthalmitis/keratitis and endocarditis. First, *S. aureus* can rapidly escape from cells via apoptosis. Wesson et al. [87] showed that an *agr* and *sarA* mutant strain failed to induce the apoptosis of cultured bovine mammary epithelial cells, suggesting that the factors responsible for apoptosis are controlled by the *agr sar* system in this model. Fibronectin-binding proteins were also reported to be important during the early steps of the colonisation of the bovine mammary gland. This adhesion is controlled by *agr* [88]. Takeuchi et al. [89] detected sequence variations in *agrB* and *agrD* from *S. aureus* strains isolated from mastitic cow's milk. This was further confirmed by Gilot et al. [23]. These sequence variations defined four groups and were associated with different levels of production of alpha-toxin and protein A. Moreover, Gilot et al. [23] demonstrated a polymorphism of the *trap* gene, suggesting that, as for the *agr* groups, the RAP–TRAP system can be specifically activated by RAP of the same allelic group. This study indicates that specific genetic backgrounds of *S. aureus* strains, at least in the two-component regulatory factors, *agr* and *trap*, could be associated with particular hosts and diseases. However, no relationship was found between the *agr* and *trap* groups.

Ocular infection models have been developed in rabbits. Experimental endophthalmitis studies showed that an *agr* mutant causes less severe clinical and histological signs than the wild-type strain [90], strongly suggesting that toxin production contributes to virulence. This was further confirmed in the same model of endophthalmitis, where *agr*, but not *sarA* alone, influenced virulence. However, the double mutant had a greater effect [91]. In a rat endophthalmitis model, Giese et al. [92] reported that an *agr*/*sarA* double mutant strain induced less inflammatory cell recruitment than the wild-type strain, implying that pore-forming toxins may play a major role. A keratitis model showed that α-toxin production in conjunction with the presence of *agr* plays a role in virulence [93]. However, in this model, which was developed to evaluate the ability of mutant *S. aureus* strains to invade human corneal epithelial cells, the *agr*/*sarA* mutation did not reduce invasion, which appeared to be essentially driven by fibronectin-binding proteins.

Such discrepancies may be due to differences in procedures and bacterial environments [94]. More recently, we investigated the influence of antibiotics on the expression of *agr*, *sarA* and bicomponent leucotoxins. For this, we used a competitive RT-PCR test allowing the semi quantification of mRNAs produced by colony-forming units (CFU) in a controlled model of rabbit experimental endophthalmitis. Serious clinical signs of infection appeared at bacterial densities of just 10^6 CFU per ml of rabbit vitreous [95]. At these bacterial densities, we were able to detect expression of the Panton-Valentine leucocidin gene and of the gamma-hemolysin and SarA genes, but not that of *agr*. This is consistent with the results obtained by Ohlsen et al. [41] showing that in some culture conditions (e.g. at 42 °C), RNAIII may not be highly expressed, whereas the α-toxin gene is strongly expressed. In our experience, clindamycin and moxifloxacin, a 3rd generation fluoroquinolone, do not enhance virulence factor expression and thus do not generate the risk of toxin-induced lesions.

In the rabbit endocarditis model, the *sarA* and *agr*/*sarA* mutant strains showed reduced infectivity and decreased levels of hemolysin and fibronectin-binding protein compared to the wild-type strains [96]. However, the same authors identified another locus distinct from *agr* and *sarA* that modulates the expression of genes encoding toxins, protein A and fibronectin-binding protein and decreases infectivity in endocarditis. In this model, RNAII is transcribed before RNAIII, which is expressed in a time- and cell density-dependent manner. However, in this model, each animal was inoculated with at least 10^7 CFU [97]. The capsular type 5 polysaccharide is an important virulence factor in the establishment of *S. aureus* infection, due to its antiphagocytic properties. In a rabbit endocarditis model, van Wamel et al. [98] found that SarA makes a minor contribution to *cap5* regulation, whereas *agr* positively regulates *cap5* expression. However, *cap5* expression was found to vary according to ecological niches.

The structure and function of RIP and its analogues were further evaluated to identify the critical residues for

function. This study showed that an RIP analogue in which the lysine at position 2 has been replaced by a serine and the isoleucine at position 4 had been substituted by tryptophan had a greater inhibitory effect on *S. aureus* Smith Diffuse infections in a murine model of cellulitis than did the wild type [99].

8. Perspectives for chemotherapeutic agents that target *S. aureus* virulence

Most applications of this research are turned towards improving prophylaxis and treatment of infections by neutralising virulence factor expression.

For example, Fujimoto et al. [100] showed that both *agr* and *sarA* positively regulate *lrgAB*, which controls tolerance to penicillin. SarA is thought to be a key effector in this process and to interact with specific DNA inverted repeats. Mimetic inhibitors of this interaction would be valuable auxiliaries to antibiotics, promptly reducing the expression of some virulence factors and, thus, the aggressive potential of bacteria in some infections, but also might decrease the tolerance to penicillin by improving access to its target.

In the case of *agr*, perspectives of applications essentially focus on the potential use of modified AIPs or mimetics to inhibit the interaction of the natural ones. McDowell et al. [101] revealed that altered AIPs might become activators or inhibitors depending on modifications. Lyon et al. [102] recently showed that the N-terminus of AgrC is the AIP receptor domain. These authors [34] studied the structure of AIPs essential for their function. It appeared that the thiolactone bridge was essential, whereas the N-terminal sequence was less critical. Autoinducing peptides from the *agr* group 1 (AIP_I) and group 4 (AIP_{IV}) carry an essential endocyclic residue, whereas those from AIP_{II} contain an exocyclic one. Nevertheless, cross-inhibition between AIPs confirmed that they all use the same receptor, despite differences in activation. Precise knowledge of these interactions and the structures involved would help us to conceive new inhibitors and antibiotic auxiliaries able to prevent infections.

Its ability to inhibit protein synthesis makes RIP a potential factor for anti-staphylococcal chemotherapy. In vivo, native or synthetic RIP can prevent cellulitis caused by the *S. aureus* Smith Diffuse strain in mice [26], keratitis and osteomyelitis in rabbits, mastitis in cows and septic arthritis in mice [103]. Furthermore, immunisation against RAP decreases the incidence and severity of staphylococcal infections in a mouse model of *S. aureus* cellulitis [26]. Balaban et al. used RIP to inhibit biofilm formation inside catheters [104].

Finally, the use of antisense RNA [105] efficiently controlled the expression of a targeted gene. This approach might be interesting to inhibit RNAIII. As these molecules must have a long half-life and availability during bacterial infection and division, it would probably be more favorable to evaluate molecular mimetics, even though their toxicity to higher organisms has not yet been assessed.

9. General conclusions

The expression of *S. aureus* virulence genes is regulated by complex pathways, where *agr* and *sarA* appear to be two global systems. The *agr* system is a particular model for a chromosomal system of an activating antisense RNA, able to target major factors associated with the virulence of *S. aureus* [6,106].

The publication of the complete genomic sequences of several *S. aureus* strains and the recent development of new molecular biology tools have allowed the identification of some new genes involved in the regulation, thus increasing the complexity of the regulatory networks and the possible interactions between these factors. This complexity and specificity of the interactions are probably the keys to the accurate and pleiotropic regulation of the expression of the virulence factors according to the external environment, especially at the site of infection, thus allowing the survival of the bacterium. The *agr* locus might be regulated specifically according to the type of infection and the in vivo bacterial environment [40]. Further studies are needed to elucidate the finer details of these regulatory pathways. The competition between some molecules for their targets and their possible pleiotropic binding remains questionable for AIPs and RAP, for example. Such findings may lead to the development of new therapeutic agents.

Therefore, the use of RIP or RIP analogues as auxiliary therapeutic agents looks promising because they can block at least one of the global regulatory systems and might be useful in the treatment of infections [104].

Acknowledgements

This work was supported by grant EA-3432 from the "Direction de la Recherche et des Etudes Doctorales". Stéphane Bronner was a post-doc granted by the European project n° QLK2-CT-2002-00887. English correction was achieved by Alex Edelmann & Associates (http://www.alexedelmann.com).

References

[1] Gladstone, G.P. and Fildes, P. (1940) A simple culture medium for general use without meat extract or peptone. Br. J. Exp. Pathol. 21, 161–173.

[2] Coleman, G. (1983) The effect of glucose on the differential rates of extracellular protein and alpha-toxin formation by *Staphylococcus aureus* (Wood 46). Arch. Microbiol. 134, 208–211.
[3] Barret, J.F. and Hoch, J.A. (1998) Two-component signal transduction as a target for microbial anti-infective therapy. Antimicrob. Agents Chemother. 42, 1529–1536.
[4] Dunman, P.M., Murphy, E., Haney, S., Palacios, D., Tucker-Kellogg, G., Wu, S., Brown, E.L., Zagursky, R.J., Shlaes, D. and Projan, S.J. (2001) Transcription profiling-based identification of *Staphylococcus aureus* genes regulated by the *agr* and/or *sarA* loci. J. Bacteriol. 183, 7341–7353.
[5] Novick, R.P., Ross, H.P., Projan, S.J., Kornblum, J., Kreiswirth, B. and Moghazeh, S. (1993) Synthesis of staphylococcal virulence factors is controlled by a regulatory RNA molecule. EMBO. J. 12, 3967–3975.
[6] Morfeldt, E., Taylor, D., von Gabain, A. and Arvidson, S. (1995) Activation of alpha-toxin in *Staphylococcus aureus* by the *trans*-encoded antisense RNA, RNAIII. EMBO. J. 14, 4569–4577.
[7] Regassa, L.B., Novick, R.P. and Betley, M.J. (1992) Glucose and nonmaintained pH decrease expression of the accessory gene regulator (*agr*) in *Staphylococcus aureus*. Infect. Immun. 60, 3381–3388.
[8] Gillaspy, A.F., Patti, J.M. and Smeltzer, M.S. (1997) Transcriptional regulation of the *Staphylococcus aureus* collagen adhesin gene, *cna*. Infect. Immun. 65, 1536–1540.
[9] Bronner, S., Stoessel, P., Gravet, A., Monteil, H. and Prévost, G. (2000) Variable expression of *Staphylococcus aureus* bicomponent leucotoxins semiquantified by competitive reverse transcription-PCR. Appl. Environ. Microbiol. 66, 3931–3938.
[10] Goerke, C., Campana, S., Bayer, M.G., Döring, G., Botzenhart, K. and Wolz, C. (2000) Direct quantitative transcript analysis of the *agr* regulon of *Staphylococcus aureus* during human infection in comparison to the expression profil in vitro. Infect. Immun. 68, 1304–1311.
[11] Recsei, P., Kreiswirth, B., O'Reilly, M., Schlievert, P., Gruss, A. and Novick, R.P. (1986) Regulation of exoprotein gene expression by *agr*. Mol. Gen. Genet. 202, 58–61.
[12] Peng, H.L., Novick, R.P., Kreiswirth, B., Kornblum, J. and Schlievert, P. (1988) Cloning, characterization, and sequencing of an accessory gene regulator (*agr*) in *Staphylococcus aureus*. J. Bacteriol. 170, 4365–4372.
[13] Novick, R.P., Projan, S.J., Kornblum, J., Ross, H.F., Ji, G., Kreiwirth, B., Vandenesch, F. and Moghazeh, S. (1995) The *agr* P2 operon: an autocatalytic sensory transduction system in *Staphylococcus aureus*. Mol. Gen. Genet. 248, 446–458.
[14] Ji, G., Beavis, R. and Novick, R.P. (1995) Cell density control of staphylococcal virulence mediated by an octapeptide pheromone. Proc. Natl. Acad. Sci. USA 92, 12055–12059.
[15] Janzon, L., Lofdahl, S. and Arvidson, S. (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.
[16] Janzon, L. and Arvidson, S. (1990) The role of the δ-lysin gene (*hld*) in the regulation of virulence genes by the accessory gene regulator (*agr*) in *Staphylococcus aureus*. EMBO J. 9, 1391–1399.
[17] Zhang, L., Gray, L., Novick, R.P. and Ji, G. (2002) Transmembrane topology of AgrB, the protein involved in the post-translational modification of AgrD in *Staphylococcus aureus*. J. Bio. Chem. 38, 34736–34742.
[18] Mayville, P., Ji, G., Beavis, R., Yang, H., Goger, M., Novick, R.P. and Muir, T.W. (1999) Structure–activity analysis of synthetic autoinducing thiolactone peptides from *Staphylococcus aureus* responsible from virulence. Proc. Natl. Acad. Sci. USA 96, 1218–1223.
[19] Ji, G., Beavis, R. and Novick, R.P. (1997) Bacterial interference caused by autoinducing peptide variants. Science 276, 2027–2030.
[20] Fuqua, W.C., Winans, S.C. and Greenberg, E.P. (1994) Quorum sensing in bacteria: the LuxR-LuxI family of cell density-responsive transcriptional regulators. J. Bacteriol. 176, 269–275.
[21] Lina, G., Jarraud, S., Ji, G., Greenland, T., Pedraza, T., Etienne, J., Novick, R.P. and Vandenesch, F. (1998) Trans-membrane topology and histidine kinase activity of AgrC, the *agr* signal receptor in *Staphylococcus aureus*. Mol. Microbiol. 28, 655–662.
[22] Balaban, N. and Novick, R.P. (1995) Autocrine regulation of toxin synthesis by *Staphylococcus aureus*. Proc. Natl. Acad. Sci. USA 92, 1619–1623.
[23] Gilot, P., Lina, G., Cochard, T. and Poutrel, B. (2002) Analysis of the genes encoding the RNA III-activating components Agr and TRAP in a population of *Staphylococcus aureus* strains isolated from cows with mastitis. J. Clin. Microbiol. 40, 4060–4067.
[24] Balaban, N., Goldkorn, T., Gov, Y., Hirshberg, M., Koyfman, N., Matthews, H.R., Nhan, R.T., Singh, B. and Uziel, O. (2001) Regulation of *Staphylococcus aureus* pathogenesis via target of RNAIII-activating protein (TRAP). J. Biol. Chem. 276, 2658–2667.
[25] Korem, M., Sheoran, A.S., Gov, Y., Tzipori, S., Borovok, I. and Balalan, N. (2003) Characterization of RAP, a quorum sensing activator of *Staphylococcus aureus*. FEMS Microbiol. Lett. 223, 167–175.
[26] Balaban, N., Goldkorn, T., Nhan, R.T., Dang, L.B., Scott, S., Ridgley, R.M., Rasooly, A., Wright, S.C., Larrick, J.W., Rasooly, R. and Carlson, J.R. (1998) Autoinducer of virulence as a target for vaccine and therapy against *Staphylococcus aureus*. Science 280, 438–440.
[27] Novick, R.P., Ross, H.F., Figueiredo, A.M.S., Abramochkin, G. and Muir, T. (2000) Activation and inhibition of the staphylococcal *agr* system. Science 287, 391a.
[28] Garvis, S., Mei, J.M., Rauiz-Albert, J. and Holden, D.W. (2002) *Staphylococcus aureus svrA*: a gene required for virulence and expression of the *agr* locus. Microbiology 148, 3235–3243.
[29] Benito, Y., Kolb, F.A., Romby, P., Lina, G., Etienne, J. and Vandenesch, F. (2000) Probing the structure of RNAIII, the *staphylococcus aureus agr* regulatory RNA, and identification of the RNA domain involved in repression of protein A expression. RNA 6, 668–679.
[30] McNamara, P.J., Milligan-Monroe, K.C., Khalili, S. and Proctor, R.A. (2000) Identification, cloning, and initial characterization of *rot*, a locus encoding a regulator of virulence factor expression in *Staphylococcus aureus*. J. Bacteriol. 182, 3197–3203.
[31] Tegmark, K., Karisson, A. and Arvidson, S. (2000) Identification and characterization of SarH1, a new global regulator of virulence gene expression in *Staphylococcus aureus*. Mol. Microbiol. 37, 398–409.
[32] Schmidt, K.A., Manna, A.C., Gill, S. and Cheung, A.L. (2001) SarT, a repressor of α-hemolysin in *Staphylococcus aureus*. Infect. Immun. 69, 4749–4758.
[33] Jarraud, S., Lyon, G., Figueiredo, A.M.S., Lina, G., Vandenesch, F., Etienne, J., Muir, T.W. and Novick, R.P. (2000) Exfoliatin-producing strains define a fourth *agr* specify group in *Staphylococcus aureus*. J. Bacteriol. 182, 6517–6522.
[34] Lyon, G.J., Wright, J.S., Muir, T.W. and Novick, R.P. (2002) Key determinants of receptor activation in the *agr* autoinducing peptides of *Staphylococcus aureus*. Biochemistry 41, 10095–10104.
[35] Jarraud, S., Mougel, C., Thioulouse, J., Lina, G., Meugnier, H., Forey, F., Nesme, X., Etienne, J. and Vandenesch, F. (2002) Relationships between *Staphylococcus aureus* genetic background, virulence factors, *agr* groups (alleles), and human disease. Infect. Immun. 70, 631–641.

[36] Giraudo, A.T., Raspanti, C.G., Calzolari, A. and Nagel, R. (1994) Characterization of a Tn*551*-mutant of *Staphylococcus aureus* defective in the production of several exoproteins. Can. J. Microbiol. 40, 677–681.
[37] Giraudo, A.T., Cheung, A.L. and Nagel, R. (1997) The *sae* locus of *Staphylococcus aureus* controls exoprotein synthesis at the transcriptional level. Arch. Microbiol. 168, 53–58.
[38] Giraudo, A.T., Calzolari, A., Cataldi, A.A., Bogni, C. and Nagel, R. (1999) The *sae* locus of *Staphylococcus aureus* encodes a two-component regulatory system. FEMS Microbiol. Lett. 177, 15–22.
[39] Stock, J.B., Surette, M.G., Levit, M. and Park, P. (1995) Two-component signal transduction systems: structure–function relationships and mechanisms of catalysis. In: Two-Component Signal Transduction (Hoch, J.A. and Silhavy, T.J., Eds.), pp. 25–51. ASM Press, Washington, DC.
[40] Goerke, C., Fluckiger, U., Steinhuber, A., Zimmerli, W. and Wolz, C. (2001) Impact of the regulatory loci *agr*, *sarA* and *sae* of *Staphylococcus aureus* on the induction of α-toxin during device-related infection resolved by direct quantitative transcript analysis. Mol. Microbiol. 40, 1439–1447.
[41] Ohlsen, K., Koller, K.P. and Hacker, J. (1997) Analysis of expression of the alpha-toxin gene (*hla*) of *Staphylococcus aureus* by using a chromosomally encoded *hla*::*lacZ* gene fusion. Infect. Immun. 65, 3606–3614.
[42] Throup, J.P., Zappacosta, F., Lunsford, R.D., Annan, R.S., Carr, S.A., Lonsdale, J.T., Bryant, A.P., McDevitt, D., Rosenberg, M. and Burnham, M.K.R. (2001) The *srhSR* gene pair from *Staphylococcus aureus*: genomic and proteomic approaches to the identification and characterization of gene function. Biochemistry 40, 10392–10401.
[43] Yarwood, J.M., McCormick, J.K. and Schlievert, P.M. (2001) Identification of a novel two-component regulatory system that acts in global regulation of virulence factors. J. Bacteriol. 183, 1113–1123.
[44] Nakano, M.M., Zuber, P., Glaser, A., Danchin, A. and Hulett, F.M. (1996) Two-component regulatory proteins ResD-ResE are required for transcriptional activation of *fnr* upon oxygen limitation on *Bacillus subtilis*. J. Bacteriol. 178, 3796–3802.
[45] Ye, R.W., Tao, W., Bedzyk, L., Young, T., Chen, M. and Li, L. (2000) Global gene expression profiles of *Bacillus subtilis* grown under anaerobic conditions. J. Bacteriol. 182, 4458–4465.
[46] Wagner, G.L., Bohr, P., Wagner, P. and Petersen, L.N. (1984) Tampon-induced changes in vaginal oxygen and carbon dioxide tensions. Am. J. Obstet. Gynecol. 148, 147–150.
[47] Fournier, B., Aras, R. and Hooper, D.C. (2000) Expression of the multidrug transporter NorA from *Staphylococcus aureus* is modified by a two-component regulatory system. J. Bacteriol. 182, 664–671.
[48] Fournier, B. and Hooper, D.C. (2000) A new two-component regulatory system involved in adhesion, autolysis, and extracellular proteolytic activity of *Staphylococcus aureus*. J. Bacteriol. 183, 3955–3964.
[49] Fournier, B., Klier, A. and Rapoport, G. (2001) The two-component system ArlS–ArlR is a regulator of virulence gene expression in *Staphylococcus aureus*. Mol. Microbiol. 41, 247–261.
[50] Brunskill, E.W. and Bayles, K.W. (1996) Identification and molecular characterization of a putative locus that affects autolysis in *Staphylococcus aureus*. J. Bacteriol. 178, 611–618.
[51] Brunskill, E.W. and Bayles, K.W. (1996) Identification of LytSR-regulated genes from *Staphylococcus aureus*. J. Bacteriol. 178, 5810–5812.
[52] Groicher, K.H., Firek, B.A., Fujimoto, D.F. and Bayles, K.W. (2000) The *Staphylococcus aureus lrgAB* operon modulates murein hydrolase activity and penicillin tolerance. J. Bacteriol. 182, 1794–1801.
[53] Cheung, A.L., Koomey, J.M., Butler, C.A., Projan, S.J. and Fischetti, V.A. (1992) Regulation of exoprotein expression in *Staphylococcus aureus* by a locus (*sar*) distinct from *agr*. Proc. Natl. Acad. Sci. USA 89, 6462–6466.
[54] Cheung, A.L. and Projan, S.J. (1994) Cloning and sequencing of *sarA* of *Staphylococcus aureus* gene required for the expression of *agr*. J. Bacteriol. 176, 4168–4172.
[55] Bayer, M.G., Heinrichs, J.H. and Cheung, A.L. (1996) The molecular architecture of the *sar* locus in *Staphylococcus aureus*. J. Bacteriol. 178, 4563–4570.
[56] Manna, A. and Cheung, A.L. (2001) Characterization of *sarR*, a modulator of *sar* expression in *Staphylococcus aureus*. Infect. Immun. 69, 885–896.
[57] Deora, R., Tseng, T. and Misra, T.P. (1997) Alternative transcription factor σ^{SB} of *Staphylococcus aureus*: characterization and role in transcription of the global regulatory locus *sar*. J. Bacteriol. 179, 6355–6359.
[58] Manna, A.C., Bayer, M.G. and Cheung, A.L. (1998) Transcriptional analysis of different promoters in the *sar* locus in *Staphylococcus aureus*. J. Bacteriol. 180, 3828–3836.
[59] Bischoff, M., Entenza, J.M. and Giachino, P. (2001) Influence of a functional *sigB* operon on the global regulators *sar* and *agr* in *Staphylococcus aureus*. J. Bacteriol. 183, 5171–5179.
[60] Cheung, A.L., Chien, Y.T. and Bayer, A.S. (1999) Hyperproduction of alpha-hemolysin in a *sigB* mutant is associated with elevated SarA expression in *Staphylococcus aureus*. Infect. Immun. 67, 1331–1337.
[61] Cheung, A.L., Nast, C.C. and Bayer, A.S. (1998) Selective activation of *sar* promoters with the use of green fluorescent protein transcriptional fusions as the detection system in rabbit endocarditis model. Infect. Immun. 66, 5988–5993.
[62] Blevins, J.S., Gillaspy, A.F., Rechtin, T.M., Hurlburt, B.K. and Smeltzer, M.S. (1999) The Staphylococcal accessory regulator (*sar*) represses transcription of the *Staphylococcus aureus* collagen adhesin gene (*cna*) in an *agr*-independent manner. Mol. Microbiol. 33, 317–326.
[63] Schumacher, M.A., Hurlburt, B.K. and Brennan, R.G. (2001) Crystal structure of SarA, a pleiotropic regulator of virulence genes in *S. aureus*. Nature 409, 215–219.
[64] Chien, Y.T., Manna, A.C., Projan, S.J. and Cheung, A.L. (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.
[65] Cheung, A.L., Bayer, M.G. and Heinrichs, J.H. (1997) *sar* genetic determinants necessary for transcription of RNAII and RNAIII in the *agr* locus of *Staphylococcus aureus*. J. Bacteriol. 179, 3963–3971.
[66] Morfeldt, E., Tegmark, K. and Arvidson, S. (1996) Transcriptional control of the *agr*-dependent virulence gene regulator, RNAIII, in *Staphylococcus aureus*. Mol. Microbiol. 21, 1227–1237.
[67] Rechtin, T.M., Gillaspy, A.F., Schumacher, M.A., Brennan, R.G., Smeltzer, M.S. and Hurlburt, B.K. (1999) Characterization of the SarA virulence gene regulator of *Staphylococcus aureus*. Mol. Microbiol. 33, 307–316.
[68] Gajiwala, K.S. and Burley, S.K. (2000) Winged helix proteins. Curr. Opin. Struct. Biol. 10, 110–116.
[69] Cheung, A.L. and Zhang, G. (2001) Are the structures of SarA and SarR similar? Trends Microbiol. 9, 570–573.
[70] Liu, Y., Manna, A., Li, R., Martin, W.E., Murphy, R.C., Cheung, A.L. and Zhang, G. (2001) Crystal structure of the SarR protein from *Staphylococcus aureus*. Proc. Natl. Acad. Sci. USA 98, 6877–6882.
[71] Cheung, A.L., Schmidt, K., Bateman, B. and Manna, A.C. (2001) SarS, a SarA homolog repressible by *agr*, is an activator of

protein A synthesis in *Staphylococcus aureus*. Infect. Immun. 69, 2448–2455.
[72] Manna, A.C. and Cheung, A.L. (2003) *sarU*, a *sarA* homolog, is repressed by SarT and regulates virulence genes in *Staphylococcus aureus*. Infect. Immun. 71, 342–353.
[73] Saïd-Salim, B., Dunman, P.M., McAleese, F.M., Macapagal, D., Murphy, E., McNamara, P.J., Arvidson, S., Foster, T.J., Projan, S.J. and Kreiswirth, B.N. (2003) Global regulation of *Staphylococcus aureus* genes by Rot. J. Bacteriol. 185, 610–619.
[74] Cheung, A.L. and Zhang, G. (2002) Global regulation of virulence determinants in *Staphylococcus aureus* by the SarA protein family. Front Biosci. 7, d1825–1842.
[75] Wu, S., De Lancastre, H. and Tomasz, A. (1996) Sigma-B, a putative operon encoding alternative sigma factor of *Staphylococcus aureus* RNA polymerase: molecular cloning and DNA sequencing. J. Bacteriol. 178, 6036–6042.
[76] Kullik, I., Giachino, P. and Fuchs, T. (1998) Deletion of the alternative factor σ^B in *Staphylococcus aureus* reveals its function as a global regulator of virulence genes. J. Bacteriol. 180, 4814–4820.
[77] Voelker, U., Voelker, A., Maul, B., Hecker, M., Dufour, A. and Haldenwang, W.G. (1995) Separate mechanisms activate σ^B of *Bacillus subtilis* in response to environmental and metabolic stresses. J. Bacteriol. 177, 3771–3780.
[78] Miyazaki, E., Chen, J.M., Ko, C. and Bishai, W.R. (1999) The *Staphylococcus aureus rsbW* (*orf159*) gene encodes an anti-sigma factor of SigB. J. Bacteriol. 181, 2846–2851.
[79] Palma, M. and Cheung, A.L. (2001) σB activity in *Staphylococcus aureus* is controlled by RsbU and an additional factor(s) during bacterial growth. Infect. Immun. 69, 7858–7865.
[80] Gertz, S., Engelmann, S. and Schmid, R., et al. (2000) Characterization of the σ^B regulon in *Staphylococcus aureus*. J. Bacteriol. 182, 6983–6991.
[81] De Lencastre, H., Wu, S.W., Pinho, M.G., Ludovice, A.M., Filipe, S., Gardete, S., Sobral, R., Gill, S., Chung, M. and Tomasz, A. (1999) Antibiotic resistance as a stress response: complete sequencing of a large number of chromosomal loci in *Staphylococcus aureus* strain COL that impact on the expression of resistance to methicillin. Microb. Drug Resist. 5, 163–175.
[82] Giachino, P., Engelmann, S. and Bischoff, M. (2001) σ^B activity depends on RsbU in *Staphylococcus aureus*. J. Bacteriol. 183, 1843–1852.
[83] Vojtov, N., Ross, H.F. and Novick, R.P. (2002) Global repression of exotoxin synthesis by staphylococcal superantigens. Proc. Natl. Acad. Sci. USA 99, 10102–10107.
[84] Yarwood, J.M., McCormick, J.K., Paustian, M.L., Kapur, V. and Schlievert, P.M. (2002) Repression of the *Staphylococcus aureus* accessory gene regulator in serum and in vivo. J. Bacteriol. 184, 1095–1101.
[85] Savolainen, K., Paulin, L., Westerlund-Wikstrom, B., Foster, T.J., Korhonen, T.K. and Kuusela, P. (2001) Expression of *pls*, a gene closely associated with the *mecA* gene of methicillin-resistant *Staphylococcus aureus*, prevents bacterial adhesion in vitro. Infect. Immun. 69, 3013–3020.
[86] Prévost, G. (1999) The bi-component staphylococcal leucocidins and gamma-hemolysins (toxins). In: The Comprehensive Sourcebook of Bacterials Toxins, 2nd edn. (Alouf, J.E. and Freer, J.H., Eds.), pp. 402–418. Academic Press, Inc., London.
[87] Wesson, C.A., Liou, L.E., Todd, K.M., Bohach, G.A., Trumble, W.R. and Bayles, K.W. (1998) *Staphylococcus aureus* Agr and Sar global regulators influence internalization and induction of apoptosis. Infect. Immun. 66, 5238–5243.
[88] Lammers, A., Nuijten, P.J. and Smith, H.E. (1999) The fibronectin binding proteins of *Staphylococcus aureus* are required for adhesion to and invasion of bovine mammary gland cells. FEMS Microbiol. Lett. 180, 103–109.
[89] Takeuchi, S., Maeda, T., Hashimoto, N., Imaizumi, K., Kaidoh, T. and Hayakawa, Y. (2001) Variation of the *agr* locus in *Staphylococcus aureus* isolates from cows with mastitis. Vet. Microbiol. 79, 267–274.
[90] Booth, M.C., Atkuri, R.V., Nanda, S.K., Iandolo, J.J. and Gilmore, M.S. (1995) Accessory gene regulator controls *Staphylococcus aureus* virulence in endophthalmitis. Invest. Ophthalmol. Vis. Sci. 36, 1828–1836.
[91] Booth, M.C., Cheung, A.L., Hatter, K.L., Jett, B.D., Callegan, M.C. and Gilmore, M.S. (1997) Staphylococcal accessory regulator (*sar*) in conjunction with *agr* contributes to *Staphylococcus aureus* virulence in endophthalmitis. Infect. Immun. 65, 1550–1556.
[92] Giese, M.J., Berliner, J.A., Riesner, A., Wagar, E.A. and Mondino, B.J. (1999) A comparison of the early inflammatory effects of an *agr*-/*sar*- versus a wild type strain of *Staphylococcus aureus* in a rat model of endophthalmitis. Curr. Eye Res. 18, 177–185.
[93] O'Callaghan, R.J., Callegan, M.C., Moreau, J.M., Green, L.C., Foster, T.J., Hartford, O.M., Engel, L.S. and Hill, J.M. (1997) Specific roles of alpha-toxin and beta-toxin during *Staphylococcus aureus* corneal infection. Infect. Immun. 65, 1571–2578.
[94] Jett, B.D. and Gilmore, M.S. (2002) Host–parasite interactions in *Staphylococcus aureus* keratitis. DNA Cell Biol. 21, 397–404.
[95] Bronner, S., Jehl, F., Peter, J.D., Ploy, M.C., Renault, C., Arvis, P., Monteil, H. and Prévost, G. (2003) Moxifloxacin efficacy and vitreous penetration in a rabbit model of *Staphylococcus aureus* endophthalmitis and effect on gene expression of leucotoxins and virulence regulator factors. Antimicrob. Agents Chemother. 47, 1621–1629.
[96] Cheung, A.L., Eberhardt, K.J., Chung, E., Yeaman, M.R., Sullman, P.M., Ramos, M. and Bayer, A.S. (1994) Diminished virulence of a *sar*-/*agr*-mutant of *Staphylococcus aureus* in the rabbit model of endocarditis. J. Clin. Invest. 94, 1815–1822.
[97] Xiong, Y.Q., Van Wamel, W., Nast, C.C., Yeaman, M.R., Cheung, A.L. and Bayer, A.S. (2002) Activation and transcriptional interaction between *agr* RNAII and RNAIII in *Staphylococcus aureus* in vitro and in an experimental endocarditis model. J. Infect. Dis. 186, 668–677.
[98] Van Wamel, W., Xiong, Y.Q., Bayer, A.S., Yeaman, M.R., Nast, C.C. and Cheung, A.L. (2002) Regulation of *Staphylococcus aureus* type 5 capsular polysaccharides by *agr* and *sarA* in vitro and in an experimental endocarditis model. Microbiol. Pathog. 33, 73–79.
[99] Gov, Y., Bitler, A., Dell'Acqua, G., Torres, J.V. and Balaban, N. (2001) RNAIII inhibiting peptide (RIP), a global inhibitor of *Staphylococcus aureus* pathogenesis: structure and function analysis. Peptides 22, 1609–1620.
[100] Fujimoto, D.F., Brunskill, E.W. and Bayles, K.W. (2000) Analysis of genetic elements controlling *Staphylococcus aureus lrgAB* expression: potential role of DNA topology in SarA regulation. J. Bacteriol. 182, 4822–4828.
[101] McDowell, P., Affas, Z., Reynolds, C., Holden, M.T.G., Wood, S.J., Saint, S., Cockayne, A., Hill, P.J., Dodd, C.E.R., Bycroft, B.W., Chan, W.C. and Williams, P. (2001) Structure, activity and evolution of the group I thiolactone peptide quorum-sensing system of *Staphylococcus aureus*. Mol. Microbiol. 41, 503–512.
[102] Lyon, G.J., Wright, J.S., Christopoulos, A., Novick, R.P. and Muir, T.W. (2002) Reversible and specific extracellular antagonism of receptor-histidine kinase signaling. J. Biol. Chem. 277, 6247–6253.
[103] Balaban, N., Collins, L.V., Cullor, J.S., Hume, E.B., Medina-Acosta, E., Vieira da Motta, O., O'Callaghan, R., Rossitto, P.V., Shirtliff, M.E., da Silveira, L.S., Tarkowski, A. and Tores, J.V. (2000) Prevention of diseases caused by *Staphylococcus aureus* using the peptide RIP. Peptides 21, 1301–1311.

[104] Balaban, N., Gov, Y., Bitler, A. and Boelaert, J.R. (2003) Prevention of *Staphylococcus aureus* biofilm on dialysis catheters and adherence to human cells. Kidney Int. 63, 340–345.
[105] Franch, T., Petersen, M., Wagner, E.G., Jacobsen, J.P. and Gerdes, K. (1999) Antisense RNA regulation in prokaryotes: rapid RNA/RNA interaction facilitated by a general U-turn loop structure. J. Mol. Biol. 294, 1115–1125.
[106] Wagner, E.G., Altuvia, S. and Romby, P. (2002) Antisense RNAs in bacteria and their genetic elements. Adv. Genet. 46, 361–398.
[107] Dassy, B., Hogan, T., Foster, T.J. and Fournier, J.M. (1993) Involvement of the accessory gene regulator (*agr*) in expression of type 5 capsular polysaccharide by *Staphylococcus aureus*. J. Gen. Microbiol. 139, 1301–1306.
[108] Luong, T., Sau, S., Gomez, M., Lee, J.C. and Lee, C.Y. (2002) Regulation of *Staphylococcus aureus* capsular polysaccharide expression by *agr* and *sarA*. Infect. Immun. 70, 444–450.
[109] Vandenesch, F., Kornblum, J. and Novick, R.P. (1991) A temporal signal, independent of *agr*, is required for *hla* but not *spa* transcription in *Staphylococcus aureus*. J. Bacteriol. 173, 6313–6320.
[110] Wolz, C., Pöhlmann-Dietze, P., Steinhuber, A., Chien, Y.T., Manna, A., Van Wamel, W. and Cheung, A.L. (2000) *agr*-independent regulation of fibronectin-binding protein(s) by the regulatory locus *sar* in *Staphylococcus aureus*. Mol. Microbiol. 36, 230–243.
[111] Zhang, S. and Stewart, G.C. (2000) Characterization of the promoter elements for the Staphylococcal enterotoxin D gene. J. Bacteriol. 182, 2321–2325.
[112] Sheehan, B.J., Foster, T.J., Dorman, C.J., Park, S. and Stewart, G.S. (1992) Osmotic and growth dependent regulation of the *eta* gene of *Staphylococcus aureus*: a role for DNA supercoiling. Mol. Gen. Genet. 232, 49–57.
[113] Chan, P.F. and Foster, S.J. (1998) Role of SarA in virulence determinant production and environmental signal transduction in *Staphylococcus aureus*. J. Bacteriol. 180, 6232–6241.
[114] Reed, S.B., Wesson, C.A., Liou, L.E., Trumble, W.R., Schlievert, P.M., Bohach, G.A. and Bayles, K.W. (2001) Molecular characterization of a novel *Staphylococcus aureus* serine protease operon. Infect. Immun. 69, 1521–1527.