



# Genetic regulation of the intercellular adhesion locus in staphylococci

David Cue, Mei G. Lei and Chia Y. Lee\*

Department of Microbiology and Immunology, University of Arkansas for Medical Sciences, Little Rock, AR, USA

## Edited by:

David Heinrichs, University of Western Ontario, Canada

## Reviewed by:

Motoyuki Sugai, Hiroshima University, Japan

Ambrose Cheung, Dartmouth Medical School, USA

## \*Correspondence:

Chia Y. Lee, Department of Microbiology and Immunology, University of Arkansas for Medical Sciences, 4301 Markam Street, Slot 511, Little Rock, AR 72205, USA.  
e-mail: clee2@uams.edu

The formation of biofilms by *Staphylococcus aureus* and *Staphylococcus epidermidis* is an important aspect of many staphylococcal infections, most notably endocarditis, osteomyelitis and infections associated with indwelling medical devices. The major constituents of staphylococcal biofilms are polysaccharides, such as poly *N*-acetyl glucosamine (PIA/PNAG), cell surface and secreted bacterial proteins, and extracellular DNA. The exact composition of biofilms often varies considerably between different strains of staphylococci and between different sites of infection by the same strain. PIA/PNAG is synthesized by the products of four genes, *icaADBC*, that are encoded in a single operon. A fifth gene, *icaR*, is a negative regulator of *icaADBC*. Expression of *icaADBC* is tightly regulated, but can often be induced *in vitro* by growing staphylococci in the presence of high salt, high glucose, or ethanol. Regulation of *icaADBC* is complex and numerous regulatory factors have been implicated in control of *icaADBC*. Many of these are well known global transcriptional regulatory factors like SarA and sigmaB, whereas other regulators, such as IcaR, seem to affect expression of relatively few genes. Here, we will summarize how various regulatory factors affect the production of PIA/PNAG in staphylococci.

**Keywords:** *Staphylococcus aureus*, *Staphylococcus epidermidis*, biofilm, intercellular adhesion locus, *ica*, PIA, PNAG

*Staphylococcus aureus* is a major nosocomial and community acquired pathogen causing a diverse array of infections ranging from superficial infections of the skin and mucosa to highly invasive and potentially lethal infections. Perhaps not surprisingly, *S. aureus* encodes a large array of virulence factors that enable the organism to infect different tissues within its host. Despite the potential of *S. aureus* to cause disease, the organism asymptotically colonizes approximately one third of the adult population with the nares being the most common niche (Iwase et al., 2010). A number of *S. aureus* infections are associated with the formation of biofilms, including endocarditis, septic arthritis and osteomyelitis, and infections associated with implanted medical devices such as prosthetic heart valves, skeletal prostheses, and catheters. The formation of biofilms not only facilitates bacterial colonization of a host, but also provides resistance to antibiotics and the host immune system. Biofilms can also serve as foci of infection for metastatic spread of bacteria and release of toxins into the bloodstream (Gotz, 2002; Fitzpatrick et al., 2005; O'Gara, 2007; Otto, 2008; Boles and Horswill, 2011).

*S. epidermidis* is a human commensal and an opportunistic pathogen capable of causing disease in immunocompromised individuals. In healthy individuals, *S. epidermidis* typically causes infections only if introduced into subcutaneous tissues by some form of trauma especially in the presence of foreign bodies. *S. epidermidis* is also a common cause of biofilm-associated infections. Because it is present on skin and mucosal surfaces, the organism has the potential to be introduced into deeper tissues during the implantation of medical devices. *S. epidermidis*

is much less virulent than *S. aureus* and the capacity to form biofilms is considered the most important virulence trait of the organism (O'Gara, 2007; Otto, 2009; Fey and Olson, 2010).

## FORMATION AND COMPOSITION OF BIOFILMS

Bacterial biofilms are complex communities of organisms containing layers of bacteria within a glycocalyx. A mature biofilm contains specific three dimensional structures referred to as towers or mushrooms separated by fluid filled channels (Costerton et al., 1999; Stoodley et al., 2002). The formation of biofilms occurs in multiple stages, initial attachment, microcolony and macrocolony formation, and detachment or disassembly (Otto, 2008; Fey and Olson, 2010; Boles and Horswill, 2011). The initial attachment of staphylococci is often mediated by cell surface proteins that bind to mammalian extracellular matrix/plasma proteins such as fibrinogen, fibronectin, collagen, vitronectin, or laminin. Collectively these bacterial proteins are frequently referred to as MSCRAMMs (microbial surface components recognizing adhesive matrix molecules) (Patti et al., 1994). Staphylococci have dozens of MSCRAMMs which can be covalently or noncovalently bound to the cell surface. Many staphylococci are capable of binding directly to plastic surfaces and researchers have often measured attachment to plastic as an *in vitro* model of attachment *in vivo*. Implanted medical devices are usually coated by plasma proteins, however, possibly obviating a need to bind directly to abiotic surfaces (Tsang et al., 2008; Beenken et al., 2010).

Non-MSCRAMM, surface localized proteins can also mediate attachment. The major cell wall autolysins, AtlA, and AtlE, (Heilmann et al., 1997; Houston et al., 2011) promote binding to hydrophobic surfaces for initial attachment and possibly biofilm accumulation (Heilmann et al., 1997; Hirschhausen et al., 2010; Houston et al., 2011). Teichoic (TA) and lipoteichoic (LTA) acids can also aid in initial attachment (Qin et al., 2007). TAs and LTAs are common components of the cell envelopes of Gram-positive bacteria that often play a role in bacterial adherence to host cells. *S. aureus* strains with a mutation in the *dlt* operon or *tagO*, both involved in TA/LTA synthesis, exhibit reduced binding to polystyrene and glass or other abiotic surfaces (Gross et al., 2001; Vergara-Irigaray et al., 2008).

The formation of microcolonies and biofilm accumulation require mechanisms for intercellular aggregation of bacteria. The production of exopolysaccharides is a common and important factor in biofilm accumulation. In both *S. aureus* and *S. epidermidis*, the major exopolysaccharide produced is termed polysaccharide intercellular adhesion (PIA), also known as poly-*N*-acetyl-glucosamine (PNAG) (Mack et al., 1996). PIA/PNAG, which has a net positive charge, may promote intercellular interactions by binding to the negatively charged surfaces of bacterial cells. PIA/PNAG may or may not interact with TAs and LTAs to foster intercellular interactions (O'Gara, 2007; Vergara-Irigaray et al., 2008). PNAG has been found to be essential for biofilm formation by many strains of *S. aureus* and *S. epidermidis*. In addition to PIA/PNAG, biofilms contain bacterial proteins and DNA as essential components with the ratios of these components being variable.

A number of staphylococcal strains exhibit PIA/PNAG-independent biofilm formation. In the latter strains, secreted proteins and extracellular DNA appear to substitute for PIA/PNAG. The fibronectin-fibrinogen binding MSCRAMMs, FnbA, and FnbB (O'Neill et al., 2008) the IgG binding Spa protein (Merino et al., 2009), and the adhesin SasG (Geoghegan et al., 2010) all contribute to biofilm formation in *S. aureus*. The biofilm-associated protein (Bap) encoded by some *S. aureus* strains that cause bovine mastitis, appears absent in human isolates. Bap is important for both initial attachment and biofilm accumulation (Cucarella et al., 2004). The accumulation-associated protein (Aap) is commonly found in *S. epidermidis* isolates. Aap forms fibrillar structures on the cell surface and may facilitate intercellular interactions (Rohde et al., 2005). Bhp, a homolog of Bap, is another accumulation-associated protein produced by some *S. epidermidis* strains (Cucarella et al., 2001). At least some strains of staphylococci appear able to switch from PIA-dependent to PIA/PNAG-independent biofilm formation (Hennig et al., 2007).

A number of soluble extracellular proteins can also affect biofilm formation. Beta toxin is a *S. aureus* sphingomyelinase capable of lysing sheep erythrocytes under the appropriate assay conditions, and killing lymphocytes (Marshall et al., 2000; Huseby et al., 2007). Huseby et al. (2010) demonstrated that beta toxin promotes biofilm accumulation by forming crosslinks with itself in the presence of extracellular DNA, producing an insoluble nucleoprotein matrix. Alpha hemolysin, a small pore forming toxin, has also been shown to be required for biofilm production in the 8325-4 strain of *S. aureus*. Inactivation of the *hla*

gene, encoding alpha hemolysin, resulted in a strain capable of initial attachment but incapable of the cell to cell interactions required for biofilm accumulation (Caiazza and O'Toole, 2003).

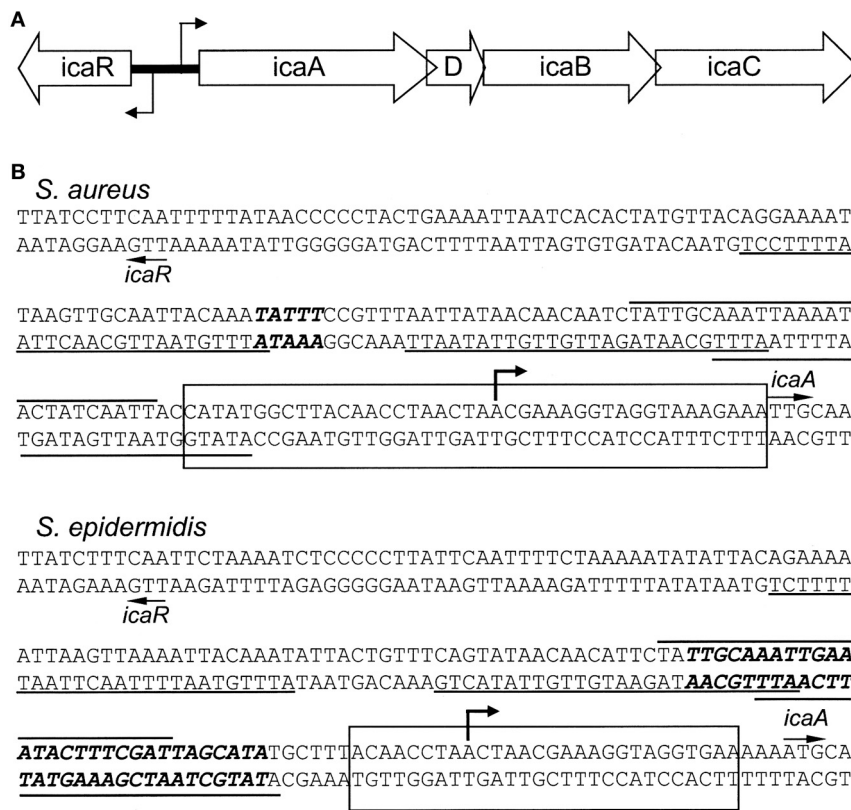
Detachment of biofilms is widely regarded as a mechanism for bacterial spread in an infected host, probably initiated by changes in pH, nutrient depletion, and waste accumulation within the biofilm. Detachment involves the degradation of the biofilm matrix by proteases and nucleases (Otto, 2008; Beenken et al., 2010; Boles and Horswill, 2011). Degradation of PNAG apparently does not occur in staphylococcal biofilms, as staphylococci do not seem to have a PNAG hydrolytic enzyme (Otto, 2009). A group of small amphiphilic  $\alpha$ -helical peptides, known as phenol-soluble modulins seem to function as surfactants, disrupting cell-to-cell interactions within the biofilm. It has been proposed that phenol-soluble modulins may play a more important role in detachment of biofilms than do proteases (Otto, 2008; Boles and Horswill, 2011).

## REGULATION OF PIA/PNAG PRODUCTION AND *icaADBC* EXPRESSION

Production of PIA/PNAG is tightly regulated and, at least *in vitro*, seems to occur primarily at the transcriptional level. Although the signals controlling PIA/PNAG production *in vivo* are not clearly defined, a number of environmental conditions affect production *in vitro*. High temperature, anaerobiosis, high osmolarity, glucose, and ethanol can all induce PIA/PNAG production although there is strain-to-strain variation in regard to which conditions result in increased PIA/PNAG production. Subinhibitory concentrations of specific antibiotics, including tetracycline, gentamicin, and the streptogramins, quinopristin and dalfoipristin, can also increase PNAG (Rachid et al., 2000b; Nuryastuti et al., 2011).

PIA/PNAG is synthesized by four proteins, IcaA, IcaD, IcaB, and IcaC, encoded by the *ica* operon (**Figure 1A**). The transmembrane proteins, IcaA, and IcaD, work in concert as an *N*-acetylglucosaminyltransferase to synthesize PNAG oligomers that are less than 20 residues in length. IcaC is a membrane protein believed to transport IcaAD-synthesized oligomers across the cell membrane. IcaC is also involved in the formation of long oligomers of PIA/PNAG. The IcaB protein, which can be found in association with the bacterial cell surface and culture supernatants, deacetylates PIA/PNAG resulting in a positively charged polymer. Deacetylation is believed to promote the interaction of PIA/PNAG with the negatively charged cell surface.

The *ica* locus was originally identified by screening a library of *S. epidermidis* transposon insertion mutants for isolates with defects in biofilm formation. A mutant with an insertion in the *ica* locus exhibited defects in biofilm formation, intercellular aggregation, and PIA synthesis (Heilmann et al., 1996a,b). The transposon insertion could be complemented by a plasmid carrying the *icaADBC* genes. Moreover, the *icaADBC* plasmid could confer a biofilm positive, aggregation, and PIA producing phenotype on the heterologous host species, *S. carnosus* (Heilmann et al., 1996b; McKenney et al., 1998). Not every isolate of *S. epidermidis* carries the *ica* locus, but *ica* genes seem to be more common in nosocomial and invasive isolates than in skin isolates (Rogers et al., 2008; Fey and Olson, 2010). It has been argued that carriage



**FIGURE 1 | (A)** Organization of the intercellular adhesion (*ica*) operon in *S. aureus* and *S. epidermidis*. The bent arrows indicate the transcriptional start sites. **(B)** The *icaR*-to-*icaA* intergenic regions. The start sites of *icaR* and *icaA* are indicated by arrows. The putative binding sites for the SarA protein are underlined or overlined (Tormo et al., 2005). The bent arrow indicates the *icaADBC* transcription start site determined for *S. epidermidis* RP62A; (Heilmann et al., 1996b; Mack et al., 2000). Top: Sequence of the *S. aureus* NCTC 8325 *ica* locus (Gillaspy et al., 2006). The

bold, italicized nucleotides indicate base pairs deleted in *S. aureus* MN8m that resulted in PIA/PNAG overproduction (Jefferson et al., 2003). The rectangle indicates the region bound by IcaR in DNase I protection experiments (Jefferson et al., 2003). Bottom: Sequence of *S. epidermidis* RP62A *ica* locus (Heilmann et al., 1996b). The bold, italicized nucleotides represent the highest affinity TcaR binding site (Chang et al., 2010). The rectangle indicates the IcaR binding site (Jeng et al., 2008).

of *icaADBC* may actually be detrimental to the survival of skin isolates of *S. epidermidis* (Rogers et al., 2008).

Subsequent to its discovery in *S. epidermidis*, the *ica* locus was found in *S. aureus* and appears to be present in nearly all isolates of the latter (Cramton et al., 1999). The *S. aureus* *ica* genes are organized as in *S. epidermidis* and the encoded proteins share from 79% to 89% similarity and 62–78% identity. The cloned *S. aureus* genes could confer biofilm production, PNAG synthesis and *N*-acetylglucosaminyltransferase activity to a *S. aureus* *ica* mutant and to *S. carnosus*.

The regulation of *icaADBC* expression is mediated by a number of regulatory factors (Table 1). These factors include global regulatory proteins such as SarA and  $\sigma^B$ , as well as factors like IcaR and TcaR which seem to regulate relatively few genes. Some factors regulate *icaADBC* expression directly (e.g., IcaR) whereas regulation by other proteins seems to be indirect (e.g.,  $\sigma^B$ ). Notably, mechanisms governing *ica* expression often vary not only between different species of staphylococci, but also between different strains of the same species. It is also worth noting that different laboratories induce *ica* expression and measure biofilm formation under a variety of different conditions. For example,

most studies utilize standard 96 well microtiter plates to assay biofilm production *in vitro*. Some researchers use untreated plates whereas others coat their plates with serum prior to adding bacteria to the wells. Proponents of the latter method argue that serum coating more closely approximates *in vivo* conditions. These variations in assay conditions can complicate comparisons of results from different laboratories. Moreover, no *in vitro* conditions or animal model can replicate the environment of an infected human host. Despite these limitations, the studies cited below have established the importance of the *ica* genes in biofilm formation by staphylococci. Here, we will summarize what is known about various regulators of the *ica* locus.

## REGULATORY FACTORS AFFECTING *ica* EXPRESSION

### *IcaR*

The *ica* locus contains the same five known genes in both *S. aureus* and *S. epidermidis* (Figure 1A). IcaADBC are encoded by a single transcript that initiates 29 bp upstream of the *icaA* start codon in *S. epidermidis* strain RP62A (Accession number U43366) (Heilmann et al., 1996b; Mack et al., 2000). The fifth gene, *icaR*, is transcribed divergently from the other *ica* genes. The

**Table 1 | Regulatory proteins affecting *icaADBC* expression in staphylococci.**

Regulatory factor	Overall effect on <i>icaADBC</i> transcription	Probable mechanism	Reference
Sa IcaR	Negative	Direct binding to <i>icaADBC</i> promoter	Conlon et al. (2002)
Se IcaR			Jefferson et al. (2003)
Sa SarA	Positive	Direct binding to <i>icaADBC</i> promoter	Valle et al. (2003)
Se SarA			Tormo et al. (2005)
Se SarX	Positive	Direct binding to <i>icaADBC</i> promoter	Rowe et al. (2010)
Se SarZ	Positive	Unknown	Wang et al. (2008)
Sa TcaR	Negative	Direct binding to <i>icaADBC</i> promoter	Jefferson et al. (2004)
Se TcaR			Chang et al. (2010)
Sa $\sigma^B$	Variable depending on study	Indirect	See text
Se $\sigma^B$			
Sa Rbf	Positive	Repression of <i>icaR</i> through a hypothetical regulator	Cue et al. (2009)
Se Rbf			Rowe (2010)
Se LuxS	Negative	Unknown	Xu et al. (2006)
Sa Spx	Negative	Upregulation of <i>icaR</i>	Pamp et al. (2006)
Se Spx	Negative	Unknown, but not through <i>icaR</i>	Wang et al. (2010)
Sa SrrAB	Positive	Direct binding to <i>icaADBC</i> promoter	Ulrich et al. (2007)
Se Ygs	Positive	Unknown	Wang et al. (2011)
Sa GdpS	Positive	Unknown	Holland et al. (2008)
Se Gdps			Tu Quoc et al. (2007)
Sa CcpA	Positive	Indirect, see text	Seidl et al. (2008)
Se CcpA			Sadykov et al. (2011)

start codons of *icaA* and *icaR* are separated by approximately 163 bp of DNA (Conlon et al., 2002).

IcaR is an approximately 22 kDa protein of the TetR family of transcriptional regulators, (Conlon et al., 2002; Jeng et al., 2008). Amino acid sequence alignments first suggested that *icaR* might encode a transcriptional regulator (Ziebuhr et al., 1999; Rachid et al., 2000a). Conlon et al. (2002) inactivated *icaR* in strain CSF41498, a clinical isolate of *S. epidermidis* that produced a weak biofilm when grown in BHI broth at 37°C. Insertional inactivation of *icaR* significantly increased *icaA* expression, indicating that *icaR* may function as a repressor of *icaADBC*. Transcription of the *icaR* gene was unaffected in *icaR* mutants indicating that *icaR* is not autoregulated, a trait that is conserved in *S. aureus* (Jefferson et al., 2003). In CSF41498, transcription of *icaA* was inducible by high NaCl, high glucose, or ethanol, whereas *icaR* expression was reduced by ethanol, but was unaffected by NaCl or glucose. Expression of *icaA* was unaffected by ethanol in CSF41498 *icaR::Erm<sup>r</sup>*, but was increased by NaCl or glucose in the same strain. It was concluded that regulation of *ica* expression by ethanol was *icaR*-dependent, whereas regulation by NaCl-glucose was *icaR*-independent. IcaR provided in *trans* was able to complement the *icaR::Erm<sup>r</sup>* mutation and repress transcription of *icaA*. Complementation of *icaR* was modulated by ethanol.

The *icaR* gene has also been shown to be a negative regulator of *icaADBC* in *S. aureus*. The predicted *S. aureus* 8325-4 IcaR protein has 65.6% identity and 90.9% similarity with IcaR from *S. epidermidis* RP62A (Heilmann et al., 1996b; Mack et al., 2000). Jefferson et al. (2003) demonstrated that IcaR can bind to a DNA region immediately 5' to *icaA* and that a short nucleotide sequence in the *icaA-icaR* intergenic region could affect expression of *icaADBC*. A spontaneous mutant of *S. aureus*

MN8, called MN8m, was isolated which exhibited constitutive hyperproduction of PIA/PNAG and enhanced biofilm formation (McKenney et al., 1999; Jefferson et al., 2003). The mutation in MN8m responsible for hyperproduction of PIA/PNAG was determined to be a 5 bp (TATTT) deletion within the *icaA-icaR* intergenic region (**Figure 1B**). The 5 bp deletion increased *icaADBC* transcription but had no effect on *icaR* expression. Substitution of the TATTT sequence with ATAAA resulted in the same phenotype as the original deletion.

DNase I protection experiments did show that recombinant IcaR protected a 42 bp region upstream of the *icaA* gene (**Figure 1B**) (Jefferson et al., 2003). The TATTT sequence, however, played no role in IcaR binding. The latter result seemed to indicate that another DNA binding protein utilizes the TATTT sequence to regulate *icaADBC* expression. It was postulated that deletion of the TATTT sequence might alter an intrinsic bend in *ica* DNA, but this possibility was not directly tested. Interestingly, the TATTT sequence lies between two putative binding sites for SarA protein which is a positive activator of *icaADBC* (Tormo et al., 2005). Thus it is possible that the 5 bp deletion affects SarA binding to the *ica* intergenic region. Precisely how the deletion affects *icaADBC* transcription has not yet been determined, however.

Subsequent work by these same authors demonstrated that *icaR* is a repressor of *ica* transcription and that the protein is functional in MN8 and MN8m (Jefferson et al., 2004). Deletion of *icaR* increased *icaADBC* expression by 100-fold and PNAG production by 10-fold. PIA/PNAG production by strain MN8 requires exogenous glucose, whereas MN8 *icaR* overproduces PNAG in the absence of glucose, leading the authors to conclude that in *S. aureus*, upregulation of PNAG by glucose is at least in



part due to alleviation of IcaR-mediated repression of *icaADBC*. Recall that glucose reportedly did not affect *icaR* expression in *S. epidermidis* CSF41498 (Conlon et al., 2002). Deletion of the *icaR* gene in *S. aureus* 8325-4 also resulted in hyperexpression of *icaADBC* and increased PNAG production (Cue et al., 2009). As described below, several different regulators appear to affect *icaADBC* expression by repression or upregulation of *icaR*.

The crystal structure of IcaR from *S. epidermidis* was recently reported (Jeng et al., 2008). Like other proteins in the TetR family, IcaR is primarily  $\alpha$ -helical. Three  $\alpha$  helices form an N-terminal DNA binding domain with  $\alpha$  helices two and three forming a helix-turn-helix motif. The native IcaR protein exists primarily as a homodimer. Dimerization is mediated by a large helix bundle formed by  $\alpha$  helices 4–9 near the C-terminus of IcaR. Electromobility shift assays (EMSAs) performed with recombinant IcaR revealed a 28 bp *ica* operator centered 17/18 nucleotides 5' to the *icaA* start codon. This agrees well with the localization of the IcaR binding site in the *S. aureus* operator (Jefferson et al., 2003). Two IcaR dimers bind cooperatively to the operator with the two dimers binding to opposite faces of the DNA. It was proposed that the binding of one *icaR* dimer may alter the DNA conformation thereby allowing binding of a second dimer (Jeng et al., 2008).

The ability of certain antibiotics to inhibit IcaR binding to DNA was also investigated, in part, because DNA binding by some members of the TetR family has been shown to be inhibited by certain antibiotics. Tetracycline, which can induce *ica* expression in *S. epidermidis*, did not affect DNA binding by IcaR, however, two aminoglycoside antibiotics, streptomycin and gentamicin, were shown to inhibit IcaR binding to DNA, presumably by directly altering the conformation of the protein (Rachid et al., 2000b; Jeng et al., 2008).

### The Sar Protein Family

The Sar (staphylococcal accessory regulator) family of proteins is composed of at least 11 different proteins some of which (e.g., SarA and SarR, SarX) are found in both *S. aureus* and *S. epidermidis*. The various Sar proteins have been categorized as fitting into one of three subfamilies (Cheung et al., 2008). The first subfamily, which includes SarA and SarX, are generally small, about 15 kDa, basic proteins with a single DNA binding domain that probably bind DNA as homodimers. Proteins in the second subfamily have two homologous DNA binding domains and likely bind DNA as monomers. The final subfamily is comprised of proteins that seem closely related to MarR protein (Liu et al., 2001, 2006; Manna and Cheung, 2001; Li et al., 2003; Ballal and Manna, 2009).

**SarA.** SarA is arguably the most well studied of the various Sar proteins. The SarA protein is a 124 amino acid residue protein that has a calculated PI of around 9. SarA is a dimeric protein with a central core region comprised of a winged-helix DNA binding domain where the helix-turn-helix domain recognizes the major groove and the winged region interacts with the minor groove. Dimerization appears to be mediated by a conserved  $\alpha$ -helical region near the N-terminus of the protein. Structure function studies have suggested that multiple SarA dimers may

bind a single target sequence and that the association of multiple dimers is fostered by  $\text{Ca}^{++}$  binding. It has been proposed that a SarA homodimer can bind a target site and recruit additional homodimers to the site (Liu et al., 2006).

The SarA protein can function as either an activator or repressor of transcription (Bayer et al., 1996; Beenken et al., 2003; Tormo et al., 2005; Oscarsson et al., 2006). SarA is a global regulatory protein affecting expression of many genes in *S. aureus* including many genes involved in pathogenesis thus making SarA a major virulence factor. Among the genes under positive regulation by SarA is the *agr* (accessory gene regulator) locus. The *agr* locus contains two divergent promoters that produce two transcripts. One transcript, RNAI, encodes four proteins that constitute a quorum sensing system. The second transcript, RNAIII, is a regulatory RNA and also encodes  $\delta$ -toxin. The *agr* system in general is involved in the switch from synthesis of cell surface proteins during exponential growth to synthesis of toxins and degradative proteins in the postexponential to stationary growth phases. Expression of *agr* can reduce the capacity of *S. aureus* to form biofilms (Vuong et al., 2000; Cafiso et al., 2007; Coelho et al., 2008; Beenken et al., 2010). Due to the fact that SarA is a positive activator of *agr*, and because *agr* can repress biofilm formation, it might be anticipated that mutation of *sarA* would increase biofilm. It appears this is not the case, however, as *sarA* mutants have a reduced capacity to form biofilms (Valle et al., 2003; Handke et al., 2007; Tsang et al., 2008; Beenken et al., 2010). This is perhaps not surprising in that SarA affects biofilm formation by affecting expression of multiple targets. For example, mutation of SarA results in increased expression of proteinases and nucleases, both of which have a negative impact on biofilm (Beenken et al., 2010). SarA also appears to enhance biofilm formation more directly by increasing *ica* expression (Valle et al., 2003).

Valle et al. (2003) screened a library of Tn917 insertion mutants to identify biofilm-defective mutants of *S. aureus*. Some of the mutants had Tn917 insertions within *sarA*. Subsequently, they deleted or insertionally inactivated *sarA* in four unrelated *S. aureus* strains, all of the mutants failed to produce a biofilm. Deletion of *agr* in the wild type strains did not affect biofilm formation, indicating that the effect of the *sarA* mutations was independent of *agr*. A series of experiments was performed to determine if increased protease production accounted for the phenotype of the *sarA* mutants. The authors concluded that enhanced proteolysis could not account for the biofilm deficient phenotype. This is somewhat at odds with some of the studies described above. In the Valle et al. (2003) study, *sarA* mutations did significantly decrease *ica* transcription and PIA/PNAG production, but this study did not determine whether SarA can bind to the *ica* promoter. Subsequent studies, however, did establish that SarA could bind the *ica* promoter (Tormo et al., 2005).

*S. epidermidis* also encodes SarA which is 84% identical to SarA from *S. aureus* (Fluckiger et al., 2005). Tormo et al. (2005) deleted *sarA* in two different clinical isolates of *S. epidermidis* and reported that both mutants were deficient in biofilm formation. PIA/PNAG production and transcription of *icaA* were both significantly reduced but not abolished in the *sarA* deleted strains. Transcription of *icaR* was unaffected by deletion of *sarA*. As has been observed for *S. aureus*, proteinase production was increased

in *sarA* mutants, which likely contributed to the mutants' inability to form biofilms. Recombinant SarA protein was shown to bind with comparable affinities to the *icaR-icaA* promoter regions of *S. aureus* and *S. epidermidis*. The *icaR-icaA* promoter regions of both species contain multiple SarA consensus binding sites (Figure 1B).

It is important to note that protein phosphorylation/dephosphorylation plays an important role in biofilm formation and SarA activity. Two *S. aureus* serine/threonine kinases, Stk1/PknB and SA0077, can both phosphorylate SarA. Phosphorylation by Stk1/PknB seems to increase the affinity of SarA for some promoters and decrease its affinity for other promoters (Didier et al., 2010). In *S. epidermidis*, Stk is required for biofilm formation and plays a major role in *icaADBC* expression (Liu et al., 2011). The *sarA*, *agr*, and *sigB* genes are all regulated by Stk1/PknB in *S. aureus* (Tamber et al., 2010), thus phosphorylation of SarA and possibly other regulatory proteins, seems likely to significantly affect *ica* expression.

**SarX.** SarX was first discovered in *S. aureus* by virtue of its homology with other Sar family proteins (Manna and Cheung, 2006). It is a 119 amino acid protein representative of the single domain class of Sar proteins. Manna and Cheung (2006) demonstrated that the SarX protein of *S. aureus* RN6390 binds to the *agr* promoter, repressing synthesis of RNAII and RNAIII, thereby indirectly repressing exoprotein synthesis. Subsequently, Rowe et al. (2010) demonstrated that SarX from *S. epidermidis* strain CSF41498 bound to its cognate *agr* promoter and repressed *agr* transcription.

Reportedly, *sarX* did not affect biofilm formation in *S. aureus* RN6390, but did promote biofilm formation by *S. epidermidis* CSF41498 in an *ica*-dependent manner (Rowe, 2010, C.Y.L. and J.P.O., unpublished data). Expression of *S. epidermidis sarX* on a multicopy plasmid not only complemented a *sarX* mutation, but also enhanced biofilm formation by the wild type strain (Rowe et al., 2010). Expression of *sarX* increased *icaA* transcription as well as PNAG production, but expression of *icaR* was unaffected by *sarX*. A purified maltose binding-SarX fusion protein bound to *ica* promoter DNA generating a ladder of protein-DNA complexes. A similar pattern was previously shown for SarX binding to the *agr* promoter region (Manna and Cheung, 2006). To account for the observed laddering, it has been suggested that either the *ica* and *agr* promoters each contain multiple SarX binding sites or that SarX oligomers form on bound DNA. Thus *sarX* appears to directly affect *icaADBC* transcription, at least in *S. epidermidis* CSF41498. Modulation of *agr* expression by SarX is apparently inadequate to affect biofilm formation in *S. aureus*.

Interestingly, *sarX* is located immediately downstream from the *rbf* gene in both *S. aureus* and *S. epidermidis* (see below) and is under positive regulation by *rbf* in *S. aureus* strains 8325-4 and UAMS-1. The *rbf* gene has been shown to upregulate biofilm formation and *icaADBC* expression and may do so, at least in part, by increasing *sarX* transcription (Lim et al., 2004; Cue et al., 2009).

**SarZ.** SarZ has also been shown to affect *ica* expression in *S. epidermidis* 1457. Wang et al. (2008) utilized a novel biofilm screening assay, involving separate and consecutive screens, to

isolate biofilm-defective mutants with Tn917 insertions. The screen resulted in the isolation of two mutants both of which had Tn917 inserted in *sarZ*. The mutants had defects in primary attachment as well as biofilm accumulation. PIA/PNAG production and *icaADBC* expression were both reduced in *sarZ* mutants. Moreover, *sarZ* was shown to contribute to virulence in both rat and mouse models of biofilm-associated infection. Microarray studies revealed that the *sarZ* regulon is comprised of at least 80 genes thus decreases in *ica* expression may not completely account for the biofilm negative phenotype of *sarZ* mutants. As an example, three genes encoding proteinases were all upregulated in the *sarZ* mutant (Wang et al., 2008). Increased proteinase activity seems likely to account, in part, for the mutant phenotype.

### TcaR

TcaR is a member of the MarR family of transcription factors and is encoded by both *S. aureus* and *S. epidermidis*. A role for TcaR in *ica* expression was first revealed by Jefferson et al. (2004) who used DNA affinity chromatography to identify *S. aureus* proteins capable of binding to a DNA fragment containing the *icaA-icaR* promoter region. Topoisomerase IV, SarA and DNA-binding protein II were also recovered in the same experiment. Purified TcaR did not produce a distinct footprint with *ica* DNA, however, and produced a ladder of complexes in EMSA experiments. These results suggested that either there are multiple TcaR binding sites in *ica* DNA or that TcaR oligomerizes once bound to DNA (Jefferson et al., 2004). Of these, the former possibility seems the most probable (Chang et al., 2010).

Northern analysis indicated that inactivation of *tcaR* increased transcription of *icaADBC* fivefold in three different strains of *S. aureus*, indicating that *tcaR* is a negative regulator of *ica*. Surprisingly, deletion of *tcaR* did not affect bacterial binding to polystyrene nor did it affect PIA/PNAG production, whereas, deletion of *icaR* affected both attachment and PIA/PNAG. When coupled with an *icaR* deletion mutation, deletion of *tcaR* increased *ica* expression fivefold over the single *icaR* mutant and 500-fold over the wild type. The *icaR* mutation alone augmented *icaA* transcription approximately 100-fold. Bacterial adherence and PNAG production were also increased in an *icaR tcaR* double mutant, relative to an *icaR* single mutant (Jefferson et al., 2004).

TcaR binds DNA as a dimer and displays non-cooperative binding to the *ica* promoter region (Chang et al., 2010). TcaR from *S. epidermidis* binds to at least one of three consecutive 33 bp pseudopalindromic sequences located immediately upstream of *icaA*. TcaR seems to have the highest affinity for the most proximal binding site which is only a few bps away from the *icaR* binding site. It is not known whether TcaR and IcaR can bind simultaneously to *ica* DNA. A number of antibiotics were shown to inhibit DNA binding by TcaR, this was believed to be due to antibiotic induced changes in the conformation of the TcaR DNA binding domain. Three aminoglycoside antibiotics, kanamycin, gentamicin, and streptomycin, were shown to inhibit DNA binding by TcaR and to promote biofilm formation by *S. epidermidis* RP62A. However other antibiotics, such as  $\beta$ -lactams, disrupted DNA binding but had no significant effect on biofilm formation. It was proposed that low concentrations of some antibiotics, by virtue of their abilities to disrupt DNA binding by TcaR and IcaR,

may derepress *icaADBC* which, in turn, would confer increased antibiotic resistance due to biofilm formation.

### $\sigma^B$

$\sigma^B$  is an alternative sigma factor found in staphylococci and other Gram-positive bacteria that plays a key role in the response to environmental stress (Conlon et al., 2004). In *S. aureus*,  $\sigma^B$  is activated by signal transduction in response to high temperature, high osmolarity, antibiotics, or extreme pH. Transcription of the *sigB* operon is driven by three distinct promoters. The first is a  $\sigma^A$ -dependent promoter that produces a transcript encoding *rsbUVW* and *sigB*. The second is a  $\sigma^B$ -dependent promoter that drives synthesis of a shorter transcript lacking *rsbU*. The third promoter is the *mazEF* promoter which drives transcription of a 3.7 kb mRNA encoding the toxin-antitoxin pair, MazEF, as well as *RsbUVW*- $\sigma^B$ . Transcription from  $p_{mazEF}$  is enhanced by heat shock and exposure to tetracycline or erythromycin (Donegan and Cheung, 2009). Full expression of *sigB* appears to require all three promoters.

The activity of  $\sigma^B$  is controlled by a network of kinases and phosphatases. In the absence of stress,  $\sigma^B$  is inactive due to its association with an anti- $\sigma$  factor, RsbW. RsbW also functions to phosphorylate and thereby inactivate the anti-anti- $\sigma$  factor RsbV. Under stress conditions, RsbU, a phosphatase, dephosphorylates RsbV. RsbV can then bind RsbW, disrupting the latter's association with  $\sigma^B$ . The released sigma factor can then associate with the core RNA polymerase (Knobloch et al., 2004).

$\sigma^B$  has been shown to regulate in excess of 200 genes, including a number of genes involved in biofilm formation (Bischoff et al., 2004; Pane-Farre et al., 2006; Nielsen et al., 2011). Rachid et al. (2000a) reported a role for  $\sigma^B$  in *S. aureus* biofilm formation in a clinical isolate and the laboratory strain RN4220. In these experiments,  $\sigma^B$  was found to be required for induction of *ica* transcription and biofilm formation in response to high NaCl. Cerca et al. (2008) looked at *icaADBC* and *icaR* expression in  $\sigma^B$ -deleted derivatives of *S. aureus* strains SA113 and Newman (Cerca et al., 2008). Surprisingly,  $\sigma^B$  was found to be a positive regulator of both *icaR* and *icaADBC*. This unexpected result was proposed to be possibly due to rather weak repression of *icaADBC* by *icaR* in the strains used in the study.

As described above, Valle et al. (2003) reported that *sarA* was critical for *ica* expression and biofilm formation in multiple, unrelated *S. aureus* clinical isolates. Additionally, they reported that mutation of  $\sigma^B$  in the same strains had no significant effect on *ica* expression or biofilm. Remarkably, inactivation of  $\sigma^B$  in *sarA* mutant strains increased PIA/PNAG production and biofilm formation relative to the single *sarA* mutant strains. The latter occurred even though *icaA* expression in *sarA*-*sigB* double mutants was significantly less than in *sarA* mutants. It was proposed that  $\sigma^B$  might upregulate expression of a factor involved in turnover of PIA/PNAG.

*S. epidermidis* also possesses a *sigB* operon similar in size, organization, and function as *sigB* in *S. aureus*. *S. epidermidis*  $\sigma^B$  has been shown to affect biofilm production both *in vivo* and *in vitro* (Knobloch et al., 2004; Handke et al., 2007; Pintens et al., 2008). RsbU was shown to function as a negative regulator of *icaADBC* in *S. epidermidis* strains 1457 and 8400 (Knobloch

et al., 2004). This effect was shown to be due to a reduction of  $\sigma^B$  expression that, in turn, increased *icaR* expression. Increased *icaR* expression decreased *icaADBC* expression and biofilm. As was observed for *S. aureus*, high NaCl concentration did not induce biofilm in a *sigB* mutant. The biofilm defect in *sigB* mutants could be overcome, however, by growth in the presence of subinhibitory concentrations of ethanol. The effect of ethanol was due to  $\sigma^B$ -independent repression of *icaR*. The  $\sigma^B$  defect could also be overcome by multiple copies of *icaADBC*. The repression of *icaR* via ethanol has been speculated to involve an unknown intermediate factor. Notably, *sarA* was expressed in *sigB* mutants via the  $\sigma^B$ -independent *sarA* promoters, P1 and P2, thus decreased *icaADBC* expression was apparently not due to loss of *SarA*.

Upregulation of biofilm by anaerobiosis also involves  $\sigma^B$ . Anaerobic activation of *icaADBC* was  $\sigma^B$ -dependent and was concomitant with  $\sigma^B$ -dependent repression of *icaR*.  $\sigma^B$  appears to play a more important role in *ica* regulation under anaerobic conditions than it does under aerobic conditions, at least in *S. epidermidis* 1457 (Cotter et al., 2009). None of the aforementioned studies found any evidence for direct regulation of *ica* genes by  $\sigma^B$  in either *S. aureus* or *S. epidermidis*. This coupled with the lack of a  $\sigma^B$  consensus promoter sequence in the *ica* intergenic region, implies  $\sigma^B$  regulation is indirect.

The role of  $\sigma^B$  in biofilm expression is further complicated by the fact that  $\sigma^B$  can also activate *ica*-independent biofilm formation.  $\sigma^B$  has also been shown to function in *ica*-independent biofilm formation in *S. aureus* USA300 LAC, a CA-MRSA isolate (Lauderdale et al., 2009). In general,  $\sigma^B$  can promote biofilm by repressing the production of proteases and toxins, an effect that is manifest through decreasing expression of RNAPIII as well as through positive activation of *sarA*. In USA300 LAC, loss of  $\sigma^B$  increased the level of RNAPIII and mutation of *agr* restored biofilm formation in a *sigB* mutant. The addition of proteinase inhibitors to growth media also restored biofilm in a  $\sigma^B$  deficient strain. Thus another role of  $\sigma^B$  appears to be repression of *agr* that, in turn, represses the production of proteinases that are involved in the disassembly of biofilm.

### *Rbf*

Rbf (regulator of biofilm) is a transcriptional regulatory protein found to play an important role in biofilm formation in both *S. aureus* 8325-4 and *S. epidermidis* CSF 41498 (Lim et al., 2004; Rowe, 2010). Rbf is a member of the AraC/XylS family of transcriptional regulators, a family that bears a highly conserved 100 amino acid region forming a dual, helix-turn-helix DNA binding motif. The dual helix-turn-helix is usually localized in the C-terminal region of a protein (Gallegos et al., 1997). The two helix-turn-helix domains within a monomer are believed to interact with consecutive major grooves of DNA, thus their binding sites are typically longer than they are for classical HTH proteins (Schleif, 2010). There are hundreds of different AraC/XylS-like proteins, many of which have been identified in sequence data bases by virtue of possessing the dual HTH motif (Gallegos et al., 1997). *S. aureus* is predicted to encode at least 6 AraC/XylS-like proteins at least two of which, Rbf and Rsp, regulate biofilm formation (Lim et al., 2004; Lei et al., 2011).



Typical AraC/XylS proteins are relatively small, 250–300 amino acids long, and many have effector binding sites in the N-terminal domains of the proteins. The effector binding sites and DNA binding regions are typically separated by a linker region. The effector binding domain often regulates the DNA binding activity (Gallegos et al., 1997). For example, DNA binding to the *araBAD* promoter by the AraC protein of *E. coli*, is affected by binding of arabinose and some other sugars. In the presence of arabinose, AraC binds to two half-sites, called I1 and I2, which lie just upstream of *araBAD*. In this conformation AraC can interact with RNA polymerase and promote transcription of *araBAD*. In the absence of arabinose, AraC binds to the I1 half-site and to a third half-site, O1, which lays 210 bp upstream of I1. In this conformation a DNA loop is formed and transcription of *araBAD* is repressed (Schleif, 2010).

Although Rbf protein has the conserved DNA binding motif, it is an atypical AraC-like protein. First, it is significantly larger than most other AraC proteins, approximately 716 amino acid residues in *S. aureus* 8325-4. Additionally, the DNA binding motif of Rbf is located near the N-terminus of the protein. Expression of *rbf* is likely to be complex as the promoter-regulatory region contains putative binding sites for  $\sigma^B$  and *saeR* (C.Y.L. unpublished). The *rbf* gene was originally identified by screening of a transposon insertion library of strain 8325-4 for biofilm-defective mutants (Lim et al., 2004). Loss of *rbf* led to a defect in biofilm formation in response to high NaCl and glucose, but did not affect ethanol-induced biofilm. Inactivation of *rbf* did not affect initial attachment of staphylococci to polystyrene but did severely inhibit multicellular aggregation. Extensive macrocellular clumping was observed when Rbf from either *S. aureus* or *S. epidermidis* was expressed from a multicopy plasmid (Lim et al., 2004; Rowe, 2010). Additionally, multicopy *rbf* increased biofilm formation in *S. aureus* via increasing intercellular aggregation. The protein was also found to play a significant role in biofilm formation *in vivo* (Luong et al., 2009).

In a subsequent study, microarray experiments were performed to determine the *rbf*-regulon in a clinical isolate of *S. aureus* strain UAMS-1 (Cue et al., 2009). Expression of Rbf from a multicopy plasmid was found to increase expression of six genes and reduce expression of 35 genes. A number of the *rbf*-regulated genes could, potentially at least, affect biofilm formation. The *tagB* gene, which encodes teichoic acid biosynthesis gene B, is upregulated by *rbf*. Four genes likely to affect cell lysis and DNA release in biofilms, *lytSR*, *lrgAB* are all repressed by *rbf*. The *lytSR* genes encode a two component system that upregulates *lrgAB*. The *lrgA* gene product function as an antiholin that can inhibit cell lysis and the release of DNA into the environment (Sadykov and Bayles, 2011). Thus by inhibiting *lytSR* expression, Rbf would be predicted to increase the level of extracellular DNA in biofilm. SarX, a protein observed to enhance *icaADBC* expression in *S. epidermidis* (Rowe et al., 2010) is regulated by *rbf* in *S. aureus* UAMS-1. The gene encoding KdpD, a histidine kinase affecting *luxS* expression, is also regulated by *rbf* (Cue et al., 2009).

The microarray experiments performed with UAMS-1 produced two surprising results. The first was that deletion of *rbf* had no effect on gene expression. This was apparently due to the fact

that the *rbf* gene in UAMS-1 has a 2 bp insertion near the N-terminal coding region. Thus, although *rbf* is transcribed, no Rbf would be synthesized. The second surprise was that multi-copy *rbf* increased *icaADBC* transcription about five to sixfold. This was surprising due to the fact that Lim et al. (2004) reported that expression of an *icaA*-xylE fusion was unaffected by inactivation of *rbf* in 8325-4. Real time quantitative PCR experiments, as well as PIA/PNAG, assays confirmed that *rbf* does positively regulate *icaADBC* transcription in 8325-4 and UAMS-1. The reason Rbf failed to increase transcription of the *icaA*-XylE fusion is unclear at this time.

The microarray experiments also revealed that *rbf* can reduce *icaR* transcription, a finding confirmed by qRT-PCR experiments. Thus, it appears that *rbf* activates *icaADBC* expression, at least in part, via inhibiting expression of *icaR*. Most AraC/XylS proteins act as activators of transcription and some, such as AraC, also act as repressors (Gallegos et al., 1997). It is possible that *rbf* affects *ica* by direct activation of *icaADBC*, in addition to repression of *icaR*. Experiments to test for direct binding of Rbf to the *ica* promoter have yielded only negative results, suggesting that *rbf* regulation may be indirect (Cue et al., 2009; Rowe, 2010). Recombinant Rbf also failed to bind to the promoter regions of other genes, (i.e., *sarA*, *sarX*, *sarZ*, *spx*, and *srrA*) that regulate *ica* (Rowe, 2010).

Rbf in *S. epidermidis* CSF41498 is 46% homologous and 65% similar to Rbf from *S. aureus* with the highest similarity being in the putative DNA-binding domains (Rowe et al., 2010). Expression of the CSF414498 *rbf* gene in *S. aureus* increased macroscopic cell clumping, biofilm formation and *icaA* expression. Site-specific mutagenesis of the *rbf* DNA-binding domain, resulted in the loss of Rbf-induced cell clumping and biofilm. As was observed for 8325-4, mutation of *rbf* in CSF41498 reduced biofilm formation in response to high NaCl and glucose, but not in response to ethanol. Initial attachment by *S. epidermidis* was unaffected by *rbf*. Interestingly, the cloned *rbf* gene did not fully complement the biofilm defect of CSF41498 *rbf*, leading Rowe (2010) to propose that overexpression of Rbf may have negative effects on biofilm formation. It has been demonstrated that Rbf could bind specifically to the *sarR* promoter of *S. epidermidis* and that *rbf* had a modest effect on *sarR* transcription in stationary phase cultures. Furthermore, it was found that SarR protein could bind specifically to the *ica* promoter. While these data support a model where Rbf could regulate *ica* expression through SarR, mutation of *sarR* had no significant effect on *icaADBC* expression or biofilm formation, thus the significance of the DNA binding studies is unclear at present (Rowe, 2010).

### LuxS

LuxS is part of a quorum sensing system found in numerous species of Gram-negative and Gram-positive bacteria, including *S. aureus* and *S. epidermidis* (Doherty et al., 2006; Li et al., 2008). LuxS is required for the synthesis of autoinducer 2 (AI-2) a family of small, diffusible compounds that can penetrate cell membranes. Unlike other quorum-sensing systems *luxS* is not species-specific, rather, AI-2 produced by one species can affect gene expression in multiple bacterial species.



Several groups have determined that *luxS* is a negative regulator of biofilm formation in *S. epidermidis* (Kong et al., 2006; Xu et al., 2006; Li et al., 2008). AI-2 can be found in culture supernatants of *S. epidermidis* and is secreted optimally during log and early stationary phases of growth. AI-2 present in culture supernatants of *S. epidermidis* can activate expression of AI-2 responsive genes in *E. coli* DH5 $\alpha$  (Xu et al., 2006).

Deletion of *lux* in *S. epidermidis* strain 1457 was found to enhance biofilm formation. This effect seemed largely due to changes in *ica* expression as transcription of *icaC* increased over fourfold and PIA/PNAG synthesis was enhanced about threefold in the *luxS* strain. Expression of *ica* returned to the wild type level in a complemented strain and when exogenous AI-2 was added to cultures of the *luxS* mutant. Whether *icaR* expression was affected by deletion of *luxS* was not reported. The *luxS* mutant was found to be more virulent than the wild type strain in a rat intravascular, central-venous-catheter-associated model, presumably due to increased PIA/PNAG production by the mutant strain (Xu et al., 2006).

While *luxS* appears to be an important *ica* regulator in *S. epidermidis*, the role of *luxS* in *S. aureus* biofilm formation is less clear. Doherty et al. (2006) found no role for *luxS* in the expression of virulence traits in strain RN6390, including biofilm formation. LuxS is reportedly inactivated by serine/threonine phosphorylation in *S. aureus*, but the effects on biofilm formation have not been reported (Cluzel et al., 2010). It has been reported, however, that a furanone derived from a marine algae could promote biofilm formation by *S. aureus* strain Newman and *S. epidermidis* strain 1457. In *S. epidermidis*, enhanced biofilm formation correlated with reduced *luxS* expression and increased PIA/PNAG production. It was not reported whether the furanone affected biofilm formation by the same mechanism in *S. aureus* (Kuehl et al., 2009).

### Spx

The Spx protein is a global transcriptional regulator that is itself subject to regulation by the energy-dependent ClpXP proteinase complex in a number of Gram-positive bacteria. Spx appears to function in *S. aureus* in much the same manner as Spx in *Bacillus subtilis*. In the latter organism, Spx acts as both a transcriptional activator and a repressor. Spx binds directly to the  $\alpha$  subunit of RNA polymerase thereby potentially blocking the interaction between RNA polymerase and other transcription factors. Spx can also directly affect promoter recognition by RNA polymerase (Nakano et al., 2010). Spx is a redox sensitive regulator that can activate genes, such as those encoding thioredoxin and thioredoxin reductase, important in the cellular response to oxidative stress. The N-terminal region of Spx contains two cysteine residues that form an intramolecular disulfide bond under thiol-oxidizing conditions. Oxidized Spx can associate with RNA polymerase and direct transcription of select *B. subtilis* genes (Nakano et al., 2003, 2005).

In *S. aureus* 8325-4, Spx plays an important role in response to stress as a *spx* mutant is hypersensitive to high and low temperatures, high osmolarity, and oxidative stress. Transcription of *txrB*, the gene encoding thioredoxin reductase, requires *spx* in strain 8325-4. ClpP and Spx also affect biofilm formation. In a *spx*

mutant, initial attachment, cell aggregation and biofilm formation were all enhanced. Transcription of *icaADBC* was increased in *spx* mutants while *icaR* transcription was decreased. Thus the normal role of Spx with regard to *ica* expression is to repress *icaADBC* via enhancement of *icaR* transcription. Precisely how Spx enhances *icaR* expression is unclear. It should be noted that, while an *S. aureus* *spx* null mutant could be constructed, the mutant exhibited growth defects even in the absence of stress (Pamp et al., 2006).

In *S. epidermidis*, *clpP* mutants reportedly accumulate high levels of Spx and exhibit defects in initial attachment and biofilm formation (Wang et al., 2010). The *spx* gene may be essential in *S. epidermidis* as knockout of the gene was not achieved in *S. epidermidis* 1457. However, a knockdown plasmid construct carrying an antisense *spx* RNA could promote biofilm formation, *icaADBC* transcription and PIA production. Unlike the case with *S. aureus*, *spx* did not affect expression of *icaR* nor did *spx* affect initial attachment. *S. aureus* and *S. epidermidis* also differ in that the *S. epidermidis* Spx does not regulate *txrB* (Wang et al., 2010).

### SrrAB

SrrAB is a two-component regulatory system responsive to anaerobiosis (Ulrich et al., 2007). An *srrAB* mutant of *S. aureus* strain SA113 exhibited downregulation of *icaA* transcription and PIA/PNAG expression under anaerobic condition. SrrAB did not affect *icaR* expression. Phosphorylated SrrA protein bound to a 100 bp DNA segment immediately upstream of the *icaADBC* promoter. SrrAB is important for anaerobic growth and protection of staphylococci from killing by neutrophils under anaerobic conditions (Ulrich et al., 2007).

### Ygs

Ygs is a general stress response protein identified by transposon mutagenesis of *S. epidermidis* strain 1457. Strains with mutation of *ygs* show decreased survival upon exposure to a variety of stressful conditions including high temperature, high osmolarity, pH, and ethanol exposure. Loss of *ygs* disrupted biofilm formation but not primary attachment. The biofilm defect seemed to be due to decreased *icaADBC* expression and PIA/PNAG production in the mutant strains, but expression of *icaR* was unaffected by Ygs. Ygs also played a significant role in biofilm formation *in vivo* and pathogenesis in rats (Wang et al., 2011).

### GdpS

Holland et al. (2008) reported that a novel staphylococcal protein, GdpS (GGDEF domain protein from staphylococcus), plays a role in *ica* expression in *S. epidermidis* CSF41498. These authors identified *gdpS* by searching data bases for proteins with homology to diguanylate cyclases that bear a conserved GGDEF domain. These enzymes are responsible for the synthesis of cyclic-dimeric-GMP in many bacterial species. c-di-GMP allosterically activates enzymes involved in exopolymer synthesis. Staphylococci have only a single GGDEF domain protein, GdpS, which reportedly lacks cyclase activity (Holland et al., 2008). Despite this, *gdpS* was shown to enhance biofilm formation, *icaADBC* expression and PIA/PNAG production in media supplemented with NaCl. Expression of *icaR* was unaffected by mutation of *gdpS*. *S. aureus*

also encodes *gdpS* which is important in *icaADBC* expression (Tu Quoc et al., 2007), but like the *S. epidermidis* protein, lacks cyclase activity. It is unclear precisely how *gdpS* regulates *ica* expression.

### **CcpA, the TCA cycle and *ica* expression**

Vuong et al. (2005) noted that many of the same conditions that induce PIA/PNAG production, i.e., high osmolarity, high temperature, ethanol, etc., are also known to inhibit the tricarboxylic acid, or TCA, cycle. They proposed that *ica* expression may respond to the metabolic state of the cell via alterations in the levels of TCA cycle intermediates. They did show that PIA/PNAG production could be upregulated by exposing cultures of *S. epidermidis* to fluorocitrate, an inhibitor of the TCA cycle enzyme aconitase (citrate (isocitrate) hydroxylase). Subsequently, the same group inactivated the gene coding aconitase (*acnA*) in *S. epidermidis* 1457 to study its effect on biofilm and *ica* expression (Sadykov et al., 2008). TCA activity was blocked and *icaADBC* expression was increased by the *acnA* mutation. Inactivation of the TCA cycle increased the intracellular concentration of the immediate biosynthetic precursor of PIA/PNAG, UDP-*N*-acetylglucosamine. Moreover, transcripts of genes encoding enzymes for the synthesis of UDP-*N*-acetylglucosamine from glucose-6-phosphate were all increased. Thus a major effect of *acnA* inactivation is a rerouting of carbon into *N*-acetylglucosamine biosynthesis.

The level of *icaADBC* transcript increased dramatically as a result of *acnA* inactivation. Surprisingly, the expression of *icaR*, *sarA*, and *sigB* were all increased in the *acnA* mutant. To determine whether any of these regulators affected PIA/PNAG production in response to TCA cycle disruption, the effects of fluorocitrate on PIA/PNAG was determined for *icaR*, *sigB*, and *sarA* mutants. Fluorocitrate increased PIA/PNAG in both the *icaR* and *sigB* mutant strains, indicating that neither of these regulators responds to TCA-induced metabolic changes. Fluorocitrate did not significantly affect PIA/PNAG production in a *sarA* mutant, however, making *SarA* a candidate for a TCA cycle-responsive regulator (Sadykov et al., 2008).

Sadykov et al. (2008) noted, however, that the aconitase mutant accumulated higher levels of branched chain amino acids than the wild type strain. This result suggested that CodY, a transcriptional regulatory protein that is responsive to branched chain amino acids, could be involved in *icaADBC* regulation. The authors also noted that the carbon catabolite repression protein, CcpA, may respond to higher intracellular levels of fructose-6-phosphate and increase *icaADBC* expression. CodY and CcpA are both regulators of *icaADBC* in *S. aureus* (Majerczyk et al., 2008; Seidl et al., 2008).

As mentioned above, CcpA has been shown to be an activator of *icaADBC* in *S. aureus* (Seidl et al., 2008) and, more recently, in *S. epidermidis* (Sadykov et al., 2011). CcpA is the primary mediator of carbon catabolite repression in staphylococci and is known to function as either a repressor or activator of transcription. Repression of TCA cycle genes is a common response to high concentrations of glucose in culture media, a response that among Gram-positive bacteria is mediated by CcpA. The activity of CcpA is regulated by intracellular levels of glucose-6-phosphate and fructose-1,6-bisphosphate, both of which affect phosphorylation

of histidine-containing protein (Hpr). Phosphorylated Hpr can complex with CcpA affecting the interaction of the latter with DNA, typically causing CcpA to act as a repressor (Fujita, 2009).

Glucose can induce biofilm formation by *S. aureus* strain SA113 (Seidl et al., 2008). Induction of biofilm formation by glucose is dependent upon CcpA. Deletion of *ccpA* in both *S. aureus* SA113 and DSM20231 blocked biofilm formation but not initial attachment to polystyrene. CcpA was found to affect *icaA* transcription but was also required for expression of *cidA*. The latter is a putative holin protein that contributes to the release of bacterial DNA in biofilms (Ranjit et al., 2011). Biofilms formed by a SA113 *ccpA* mutant were more susceptible to disruption by exogenous DNase than were biofilms formed by SA113. Transcription of other regulatory genes, *sarA*, *arlRS*, *mgrA*, and *rbf*, were unaffected by deletion of *ccpA*. Based in part on the work with *S. epidermidis*, the effect of CcpA on transcription of *citZ* and *citB* was investigated. CitB is the *S. aureus* homolog of AcnA while *citZ* encodes citrate synthase. Both *citB* and *citZ* were repressed by *ccpA* in strain SA113 thereby linking CcpA with TCA cycle regulation. Based upon studies with *Bacillus subtilis* CcpA, a putative binding site for CcpA was found upstream of the *citZ* open reading frame. No such site was found upstream of *citB*. These findings suggested that CcpA may regulate *citZ* directly and *citB* indirectly (Seidl et al., 2008). Thus CcpA appears to play an important role in regulating biofilm in the presence of high glucose. The effect of CcpA on *ica* is likely indirect and a consequence of downregulation of the TCA cycle, in part, through repression of *citB* and *citZ* (Seidl et al., 2008).

CcpA has also been found to coordinate the TCA cycle and biofilm formation in *S. epidermidis* 1457 (Sadykov et al., 2008, 2011). Deletion of *ccpA* resulted in increases in aconitase and citrate synthase activity as well as *acnA* and *citZ* transcripts. CcpA proved critical for biofilm production in glucose-containing media. Deletion of *acnA* resulted in the upregulation of genes involved in PIA/PNAG synthesis including *icaD*, *glmU* (encoding glucosamine-1-phosphate *N*-acetyltransferase), *pfkA* (6-phosphofruktokinase), and *glnA* (glutamine synthetase). The increased expression of *icaD* and *pfkA* were *ccpA*-dependent and were manifest in 2 and 6 h cultures. Expression of *glmU* was similarly regulated except that expression was only evident in 6 h cultures. The *acnA* mutation increased the level of the *glnA* transcript after 6 h, but not after 2 h of incubation and was independent of CcpA. CcpA binding sites were located 5' to both the *acnA* and *glmU* genes. The authors argued that CcpA both regulates TCA cycle activity and conveys signals associated with the TCA cycle to PIA/PNAG biosynthetic genes (Sadykov et al., 2011).

### **SUMMARY**

It is obvious from the long list of factors that affect *ica* expression that regulation is extremely complex and multifactorial. This seems especially true for *S. aureus* as many isolates do not produce PIA/PNAG *in vitro* even though nearly all *S. aureus* isolates encode *icaADBC*. Moreover, the relative importance of the various factors seems to differ considerably between different strains as well as between species. In some instances, researchers have identified regulatory proteins that act directly on *ica* DNA.

However in other instances regulators appear to act indirectly, via affecting the expression or activity of hypothetical proteins that, in turn, interact with *ica* DNA. Even in cases where proteins have been shown to bind *ica* DNA, precisely how these factors regulate transcription is not completely clear. It seems highly likely that multiple regulatory factors are co-expressed during infection and we have virtually no information on how these factors

may interact with DNA and/or other macromolecules to regulate gene expression. Moreover, it remains unknown how PIA/PNAG synthesis is induced during infection.

## ACKNOWLEDGMENTS

This work was supported by grants AI37027 and AI067857 from the National Institute of Allergy and Infectious Diseases.

## REFERENCES

- Ballal, A., and Manna, A. C. (2009). Expression of the *sarA* family of genes in different strains of *Staphylococcus aureus*. *Microbiology* 155, 2342–2352.
- 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.
- Beenken, K. E., Blevins, J. S., and Smeltzer, M. S. (2003). Mutation of *sarA* in *Staphylococcus aureus* limits biofilm formation. *Infect. Immun.* 71, 4206–4211.
- Beenken, K. E., Mrak, L. N., Griffin, L. M., Zielinska, A. K., Shaw, L. N., Rice, K. C., Horswill, A. R., Bayles, K. W., and Smeltzer, M. S. (2010). Epistatic relationships between *sarA* and *agr* in *Staphylococcus aureus* biofilm formation. *PLoS One* 5:e10790. doi: 10.1371/journal.pone.0010790
- Bischoff, M., Dunman, P., Kormanec, J., Macapagal, D., Murphy, E., Mounts, W., Berger-Bachi, B., and Projan, S. (2004). Microarray-based analysis of the *Staphylococcus aureus* sigmaB regulon. *J. Bacteriol.* 186, 4085–4099.
- Boles, B. R., and Horswill, A. R. (2011). Staphylococcal biofilm disassembly. *Trends Microbiol.* 19, 449–455.
- Cafiso, V., Bertuccio, T., Santagati, M., Demelio, V., Spina, D., Nicoletti, G., and Stefani, S. (2007). *agr*-Genotyping and transcriptional analysis of biofilm-producing *Staphylococcus aureus*. *FEMS Immunol. Med. Microbiol.* 51, 220–227.
- Caiazza, N. C., and O'Toole, G. A. (2003). Alpha-toxin is required for biofilm formation by *Staphylococcus aureus*. *J. Bacteriol.* 185, 3214–3217.
- Cerca, N., Brooks, J. L., and Jefferson, K. K. (2008). Regulation of the intercellular adhesion locus regulator (*icaR*) by SarA, sigmaB, and IcaR in *Staphylococcus aureus*. *J. Bacteriol.* 190, 6530–6533.
- Chang, Y. M., Jeng, W. Y., Ko, T. P., Yeh, Y. J., Chen, C. K., and Wang, A. H. (2010). Structural study of TcaR and its complexes with multiple antibiotics from *Staphylococcus epidermidis*. *Proc. Natl. Acad. Sci. U.S.A.* 107, 8617–8622.
- Cheung, A. L., Nishina, K. A., Trotonda, M. P., and Tamber, S. (2008). The SarA protein family of *Staphylococcus aureus*. *Int. J. Biochem. Cell Biol.* 40, 355–361.
- Cluzel, M. E., Zanella-Cleon, I., Cozzone, A. J., Futterer, K., Duclos, B., and Molle, V. (2010). The *Staphylococcus aureus* autoinducer-2 synthase LuxS is regulated by Ser/Thr phosphorylation. *J. Bacteriol.* 192, 6295–6301.
- Coelho, L. R., Souza, R. R., Ferreira, F. A., Guimaraes, M. A., Ferreira-Carvalho, B. T., and Figueiredo, A. M. (2008). *agr* RNAPIII divergently regulates glucose-induced biofilm formation in clinical isolates of *Staphylococcus aureus*. *Microbiology* 154, 3480–3490.
- Conlon, K. M., Humphreys, H., and O'Gara, J. P. (2002). *icaR* encodes a transcriptional repressor involved in environmental regulation of *ica* operon expression and biofilm formation in *Staphylococcus epidermidis*. *J. Bacteriol.* 184, 4400–4408.
- Conlon, K. M., Humphreys, H., and O'Gara, J. P. (2004). Inactivations of *rsbU* and *sarA* by IS256 represent novel mechanisms of biofilm phenotypic variation in *Staphylococcus epidermidis*. *J. Bacteriol.* 186, 6208–6219.
- Costerton, J. W., Stewart, P. S., and Greenberg, E. P. (1999). Bacterial biofilms: a common cause of persistent infections. *Science* 284, 1318–1322.
- Cotter, J. J., O'Gara, J. P., Mack, D., and Casey, E. (2009). Oxygen-mediated regulation of biofilm development is controlled by the alternative sigma factor sigma(B) in *Staphylococcus epidermidis*. *Appl. Environ. Microbiol.* 75, 261–264.
- Cramton, S. E., Gerke, C., Schnell, N. F., Nichols, W. W., and Gotz, F. (1999). The intercellular adhesion (*ica*) locus is present in *Staphylococcus aureus* and is required for biofilm formation. *Infect. Immun.* 67, 5427–5433.
- Cucarella, C., Solano, C., Valle, J., Amorena, B., Lasa, I., and Penades, J. R. (2001). Bap, a *Staphylococcus aureus* surface protein involved in biofilm formation. *J. Bacteriol.* 183, 2888–2896.
- Cucarella, C., Tormo, M. A., Ubeda, C., Trotonda, M. P., Monzon, M., Peris, C., Amorena, B., Lasa, I., and Penades, J. R. (2004). Role of biofilm-associated protein Bap in the pathogenesis of bovine *Staphylococcus aureus*. *Infect. Immun.* 72, 2177–2185.
- Cue, D., Lei, M. G., Luong, T. T., Kuechenmeister, L., Dunman, P. M., O'Donnell, S., Rowe, S., O'Gara, J. P., and Lee, C. Y. (2009). Rbf promotes biofilm formation by *Staphylococcus aureus* via repression of *icaR*, a negative regulator of *icaADBC*. *J. Bacteriol.* 191, 6363–6373.
- Didier, J. P., Cozzone, A. J., and Duclos, B. (2010). Phosphorylation of the virulence regulator SarA modulates its ability to bind DNA in *Staphylococcus aureus*. *FEMS Microbiol. Lett.* 306, 30–36.
- Doherty, N., Holden, M. T., Qazi, S. N., Williams, P., and Winzer, K. (2006). Functional analysis of *luxS* in *Staphylococcus aureus* reveals a role in metabolism but not quorum sensing. *J. Bacteriol.* 188, 2885–2897.
- Donegan, N. P., and Cheung, A. L. (2009). Regulation of the *mazEF* toxin-antitoxin module in *Staphylococcus aureus* and its impact on *sigB* expression. *J. Bacteriol.* 191, 2795–2805.
- Fey, P. D., and Olson, M. E. (2010). Current concepts in biofilm formation of *Staphylococcus epidermidis*. *Future Microbiol.* 5, 917–933.
- Fitzpatrick, F., Humphreys, H., and O'Gara, J. P. (2005). The genetics of staphylococcal biofilm formation—will a greater understanding of pathogenesis lead to better management of device-related infection? *Clin. Microbiol. Infect.* 11, 967–973.
- Fluckiger, U., Ulrich, M., Steinhuber, A., Doring, G., Mack, D., Landmann, R., Goerke, C., and Wolz, C. (2005). Biofilm formation, *icaADBC* transcription, and polysaccharide intercellular adhesion synthesis by staphylococci in a device-related infection model. *Infect. Immun.* 73, 1811–1819.
- Fujita, Y. (2009). Carbon catabolite control of the metabolic network in *Bacillus subtilis*. *Biosci. Biotechnol. Biochem.* 73, 245–259.
- Gallegos, M. T., Schleif, R., Bairoch, A., Hofmann, K., and Ramos, J. L. (1997). Arac/XylS family of transcriptional regulators. *Microbiol. Mol. Biol. Rev.* 61, 393–410.
- Geoghegan, J. A., Corrigan, R. M., Gruszka, D. T., Speziale, P., O'Gara, J. P., Potts, J. R., and Foster, T. J. (2010). Role of surface protein SasG in biofilm formation by *Staphylococcus aureus*. *J. Bacteriol.* 192, 5663–5673.
- Gillaspay, A. F., Worrel, V., Orvis, J., Roe, B. A., Dyer, D. W., and Iandolo, J. J. (2006). “The *Staphylococcus aureus* NCTC 8325 genome,” in *Gram-Positive Pathogens* 2nd edn, ed V. A. Fischetti (Washington, DC: ASM Press, American Society for Microbiology), 381–412.
- Gotz, F. (2002). Staphylococcus and biofilms. *Mol. Microbiol.* 43, 1367–1378.
- Gross, M., Cramton, S. E., Gotz, F., and Peschel, A. (2001). Key role of teichoic acid net charge in *Staphylococcus aureus* colonization of artificial surfaces. *Infect. Immun.* 69, 3423–3426.
- Handke, L. D., Slater, S. R., Conlon, K. M., O'Donnell, S. T., Olson, M. E., Bryant, K. A., Rupp, M. E., O'Gara, J. P., and Fey, P. D. (2007). SigmaB and SarA independently regulate polysaccharide intercellular adhesion production in *Staphylococcus epidermidis*. *Can. J. Microbiol.* 53, 82–91.
- Heilmann, C., Gerke, C., Perdreau-Remington, E., and Gotz, F. (1996a). Characterization of Tn917 insertion mutants of *Staphylococcus epidermidis* affected in biofilm formation. *Infect. Immun.* 64, 277–282.
- Heilmann, C., Schweitzer, O., Gerke, C., Vanittanakorn, N., Mack, D., and Gotz, F. (1996b). Molecular basis of intercellular adhesion in the biofilm-forming *Staphylococcus epidermidis*. *Mol. Microbiol.* 20, 1083–1091.
- Heilmann, C., Hussain, M., Peters, G., and Gotz, F. (1997). Evidence for autolysin-mediated primary attachment of *Staphylococcus epidermidis*



- to a polystyrene surface. *Mol. Microbiol.* 24, 1013–1024.
- Hennig, S., Nyunt Wai, S., and Ziebuhr, W. (2007). Spontaneous switch to PIA-independent biofilm formation in an *ica*-positive *Staphylococcus epidermidis* isolate. *Int. J. Med. Microbiol.* 297, 117–122.
- Hirschhausen, N., Schlesier, T., Schmidt, M. A., Gotz, F., Peters, G., and Heilmann, C. (2010). A novel staphylococcal internalization mechanism involves the major autolysin Atl and heat shock cognate protein Hsc70 as host cell receptor. *Cell. Microbiol.* 12, 1746–1764.
- Holland, L. M., O'Donnell, S. T., Ryjenkov, D. A., Gomelsky, L., Slater, S. R., Fey, P. D., Gomelsky, M., and O'Gara, J. P. (2008). A staphylococcal GGDEF domain protein regulates biofilm formation independently of cyclic dimeric GMP. *J. Bacteriol.* 190, 5178–5189.
- Houston, P., Rowe, S. E., Pozzi, C., Waters, E. M., and O'Gara, J. P. (2011). Essential role for the major autolysin in the fibronectin-binding protein-mediated *Staphylococcus aureus* biofilm phenotype. *Infect. Immun.* 79, 1153–1165.
- Huseby, M. J., Kruse, A. C., Digre, J., Kohler, P. L., Vocke, J. A., Mann, E. E., Bayles, K. W., Bohach, G. A., Schlievert, P. M., Ohlendorf, D. H., and Earhart, C. A. (2010). Beta toxin catalyzes formation of nucleoprotein matrix in staphylococcal biofilms. *Proc. Natl. Acad. Sci. U.S.A.* 107, 14407–14412.
- Huseby, M., Shi, K., Brown, C. K., Digre, J., Mengistu, F., Seo, K. S., Bohach, G. A., Schlievert, P. M., Ohlendorf, D. H., and Earhart, C. A. (2007). Structure and biological activities of beta toxin from *Staphylococcus aureus*. *J. Bacteriol.* 189, 8719–8726.
- Iwase, T., Uehara, Y., Shinji, H., Tajima, A., Seo, H., Takada, K., Agata, T., and Mizunoe, Y. (2010). *Staphylococcus epidermidis* Esp inhibits *Staphylococcus aureus* biofilm formation and nasal colonization. *Nature* 465, 346–349.
- Jefferson, K. K., Cramton, S. E., Gotz, F., and Pier, G. B. (2003). Identification of a 5-nucleotide sequence that controls expression of the *ica* locus in *Staphylococcus aureus* and characterization of the DNA-binding properties of IcaR. *Mol. Microbiol.* 48, 889–899.
- Jefferson, K. K., Pier, D. B., Goldmann, D. A., and Pier, G. B. (2004). The teicoplanin-associated locus regulator (TcaR) and the intercellular adhesin locus regulator (IcaR) are transcriptional inhibitors of the *ica* locus in *Staphylococcus aureus*. *J. Bacteriol.* 186, 2449–2456.
- Jeng, W. Y., Ko, T. P., Liu, C. I., Guo, R. T., Liu, C. L., Shr, H. L., and Wang, A. H. (2008). Crystal structure of IcaR, a repressor of the TetR family implicated in biofilm formation in *Staphylococcus epidermidis*. *Nucleic Acids Res.* 36, 1567–1577.
- Knobloch, J. K., Jager, S., Horstkotte, M. A., Rohde, H., and Mack, D. (2004). RsbU-dependent regulation of *Staphylococcus epidermidis* biofilm formation is mediated via the alternative sigma factor sigmaB by repression of the negative regulator gene *icaR*. *Infect. Immun.* 72, 3838–3848.
- Kong, K. F., Vuong, C., and Otto, M. (2006). Staphylococcus quorum sensing in biofilm formation and infection. *Int. J. Med. Microbiol.* 296, 133–139.
- Kuehl, R., Al-Bataineh, S., Gordon, O., Luginbuehl, R., Otto, M., Textor, M., and Landmann, R. (2009). Furanone at subinhibitory concentrations enhances staphylococcal biofilm formation by *luxS* repression. *Antimicrob. Agents Chemother.* 53, 4159–4166.
- Lauderdale, K. J., Boles, B. R., Cheung, A. L., and Horswill, A. R. (2009). Interconnections between Sigma B, agr, and proteolytic activity in *Staphylococcus aureus* biofilm maturation. *Infect. Immun.* 77, 1623–1635.
- Lei, M. G., Cue, D., Roux, C. M., Dunman, P. M., and Lee, C. Y. (2011). Rsp inhibits attachment and biofilm formation by repressing *fnbA* in *Staphylococcus aureus* MW2. *J. Bacteriol.* 193, 5231–5241.
- Li, M., Villaruz, A. E., Vadyvaloo, V., Sturdevant, D. E., and Otto, M. (2008). AI-2-dependent gene regulation in *Staphylococcus epidermidis*. *BMC Microbiol.* 8, 4.
- Li, R., Manna, A. C., Dai, S., Cheung, A. L., and Zhang, G. (2003). Crystal structure of the SarS protein from *Staphylococcus aureus*. *J. Bacteriol.* 185, 4219–4225.
- Lim, Y., Jana, M., Luong, T. T., and Lee, C. Y. (2004). Control of glucose- and NaCl-induced biofilm formation by rbf in *Staphylococcus aureus*. *J. Bacteriol.* 186, 722–729.
- Liu, Q., Fan, J., Niu, C., Wang, D., Wang, J., Wang, X., Villaruz, A. E., Li, M., Otto, M., and Gao, Q. (2011). The eukaryotic-type serine/threonine protein kinase Stk is required for biofilm formation and virulence in *Staphylococcus epidermidis*. *PLoS One* 6:e25380. doi: 10.1371/journal.pone.0025380
- 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. U.S.A.* 98, 6877–6882.
- Liu, Y., Manna, A. C., Pan, C. H., Kriksunov, I. A., Thiel, D. J., Cheung, A. L., and Zhang, G. (2006). Structural and function analyses of the global regulatory protein SarA from *Staphylococcus aureus*. *Proc. Natl. Acad. Sci. U.S.A.* 103, 2392–2397.
- Luong, T. T., Lei, M. G., and Lee, C. Y. (2009). Staphylococcus aureus Rbf activates biofilm formation *in vitro* and promotes virulence in a murine foreign body infection model. *Infect. Immun.* 77, 335–340.
- Mack, D., Fischer, W., Krokotsch, A., Leopold, K., Hartmann, R., Egge, H., and Laufs, R. (1996). The intercellular adhesin involved in biofilm accumulation of *Staphylococcus epidermidis* is a linear beta-1,6-linked glucosaminoglycan: purification and structural analysis. *J. Bacteriol.* 178, 175–183.
- Mack, D., Rohde, H., Dobinsky, S., Riedewald, J., Nedelmann, M., Knobloch, J. K., Elsner, H. A., and Feucht, H. H. (2000). Identification of three essential regulatory gene loci governing expression of *Staphylococcus epidermidis* polysaccharide intercellular adhesin and biofilm formation. *Infect. Immun.* 68, 3799–3807.
- Majerczyk, C. D., Sadykov, M. R., Luong, T. T., Lee, C., Somerville, G. A., and Sonenshein, A. L. (2008). Staphylococcus aureus CodY negatively regulates virulence gene expression. *J. Bacteriol.* 190, 2257–2265.
- Manna, A., and Cheung, A. L. (2001). Characterization of sarR, a modulator of sar expression in *Staphylococcus aureus*. *Infect. Immun.* 69, 885–896.
- Manna, A. C., and Cheung, A. L. (2006). Expression of SarX, a negative regulator of agr and exoprotein synthesis, is activated by MgrA in *Staphylococcus aureus*. *J. Bacteriol.* 188, 4288–4299.
- Marshall, M. J., Bohach, G. A., and Boehm, D. F. (2000). Characterization of *Staphylococcus aureus* beta-toxin induced leukotoxicity. *J. Nat. Toxins* 9, 125–138.
- McKenney, D., Hubner, J., Muller, E., Wang, Y., Goldmann, D. A., and Pier, G. B. (1998). The *ica* locus of *Staphylococcus epidermidis* encodes production of the capsular polysaccharide/adhesin. *Infect. Immun.* 66, 4711–4720.
- McKenney, D., Pouliot, K. L., Wang, Y., Murthy, V., Ulrich, M., Doring, G., Lee, J. C., Goldmann, D. A., and Pier, G. B. (1999). Broadly protective vaccine for *Staphylococcus aureus* based on an *in vivo*-expressed antigen. *Science* 284, 1523–1527.
- Merino, N., Toledo-Arana, A., Vergara-Irigaray, M., Valle, J., Solano, C., Calvo, E., Lopez, J. A., Foster, T. J., Penades, J. R., and Lasa, I. (2009). Protein A-mediated multicellular behavior in *Staphylococcus aureus*. *J. Bacteriol.* 191, 832–843.
- Nakano, M. M., Lin, A., Zuber, C. S., Newberry, K. J., Brennan, R. G., and Zuber, P. (2010). Promoter recognition by a complex of Spx and the C-terminal domain of the RNA polymerase alpha subunit. *PLoS One* 5:e8664. doi: 10.1371/journal.pone.0008664.
- Nakano, S., Erwin, K. N., Ralle, M., and Zuber, P. (2005). Redox-sensitive transcriptional control by a thiol/disulphide switch in the global regulator, Spx. *Mol. Microbiol.* 55, 498–510.
- Nakano, S., Kuster-Schock, E., Grossman, A. D., and Zuber, P. (2003). Spx-dependent global transcriptional control is induced by thiol-specific oxidative stress in *Bacillus subtilis*. *Proc. Natl. Acad. Sci. U.S.A.* 100, 13603–13608.
- Nielsen, J. S., Christiansen, M. H., Bonde, M., Gottschalk, S., Frees, D., Thomsen, L. E., and Kallipolitis, B. H. (2011). Searching for small sigmaB-regulated genes in *Staphylococcus aureus*. *Arch. Microbiol.* 193, 23–34.
- Nuryastuti, T., Krom, B. P., Aman, A. T., Busscher, H. J., and van der Mei, H. C. (2011). Ica-expression and gentamicin susceptibility of *Staphylococcus epidermidis* biofilm on orthopedic implant biomaterials. *J. Biomed. Mater. Res. A* 96, 365–371.
- O'Gara, J. P. (2007). *ica* and beyond: biofilm mechanisms and regulation in *Staphylococcus epidermidis* and *Staphylococcus aureus*. *FEMS Microbiol. Lett.* 270, 179–188.
- O'Neill, E., Pozzi, C., Houston, P., Humphreys, H., Robinson, D. A., Loughman, A., Foster, T. J., and O'Gara, J. P. (2008). A novel *Staphylococcus aureus* biofilm phenotype mediated by the fibronectin-binding proteins, FnBPA and FnBPB. *J. Bacteriol.* 190, 3835–3850.
- Oscarsson, J., Kanth, A., Tegmark-Wisell, K., and Arvidson, S. (2006). SarA is a repressor of *hla* (alpha-hemolysin) transcription in *Staphylococcus aureus*: its apparent

- role as an activator of *hla* in the prototype strain NCTC 8325 depends on reduced expression of *sarS*. *J. Bacteriol.* 188, 8526–8533.
- Otto, M. (2008). Staphylococcal biofilms. *Curr. Top. Microbiol. Immunol.* 322, 207–228.
- Otto, M. (2009). *Staphylococcus epidermidis*—the “accidental” pathogen. *Nat. Rev. Microbiol.* 7, 555–567.
- Pamp, S. J., Frees, D., Engelmann, S., Hecker, M., and Ingmer, H. (2006). *Spx* is a global effector impacting stress tolerance and biofilm formation in *Staphylococcus aureus*. *J. Bacteriol.* 188, 4861–4870.
- Pane-Farre, J., Jonas, B., Forstner, K., Engelmann, S., and Hecker, M. (2006). The sigmaB regulon in *Staphylococcus aureus* and its regulation. *Int. J. Med. Microbiol.* 296, 237–258.
- Patti, J. M., Allen, B. L., McGavin, M. J., and Hook, M. (1994). MSCRAMM-mediated adherence of microorganisms to host tissues. *Annu. Rev. Microbiol.* 48, 585–617.
- Pintens, V., Massonet, C., Merckx, R., Vandecasteele, S., Peetermans, W. E., Knobloch, J. K., and van Eldere, J. (2008). The role of sigmaB in persistence of *Staphylococcus epidermidis* foreign body infection. *Microbiology* 154, 2827–2836.
- Qin, Z., Ou, Y., Yang, L., Zhu, Y., Tolker-Nielsen, T., Molin, S., and Qu, D. (2007). Role of autolysin-mediated DNA release in biofilm formation of *Staphylococcus epidermidis*. *Microbiology* 153, 2083–2092.
- Rachid, S., Ohlsen, K., Wallner, U., Hacker, J., Hecker, M., and Ziebuhr, W. (2000a). Alternative transcription factor sigma(B) is involved in regulation of biofilm expression in a *Staphylococcus aureus* mucosal isolate. *J. Bacteriol.* 182, 6824–6826.
- Rachid, S., Ohlsen, K., Witte, W., Hacker, J., and Ziebuhr, W. (2000b). Effect of subinhibitory antibiotic concentrations on polysaccharide intercellular adhesin expression in biofilm-forming *Staphylococcus epidermidis*. *Antimicrob. Agents Chemother.* 44, 3357–3363.
- Ranjit, D. K., Endres, J. L., and Bayles, K. W. (2011). *Staphylococcus aureus* CidA and LrgA proteins exhibit holin-like properties. *J. Bacteriol.* 193, 2468–2476.
- Rogers, K. L., Rupp, M. E., and Fey, P. D. (2008). The presence of *icaADBC* is detrimental to the colonization of human skin by *Staphylococcus epidermidis*. *Appl. Environ. Microbiol.* 74, 6155–6157.
- Rohde, H., Burdelski, C., Bartscht, K., Hussain, M., Buck, F., Horstkotte, M. A., Knobloch, J. K., Heilmann, C., Herrmann, M., and Mack, D. (2005). Induction of *Staphylococcus epidermidis* biofilm formation via proteolytic processing of the accumulation-associated protein by staphylococcal and host proteases. *Mol. Microbiol.* 55, 1883–1895.
- Rowe, S. E. (2010). *Contribution of Rbf and SarX to Biofilm Regulation in Staphylococcus epidermidis*. Ph.D. thesis, Dublin: University College Dublin.
- Rowe, S. E., Mahon, V., Smith, S. G., and O’Gara, J. P. (2010). A novel role for SarX in *Staphylococcus epidermidis* biofilm regulation. *Microbiology* 157, 1042–1049.
- Sadykov, M. R., and Bayles, K. W. (2011). The control of death and lysis in staphylococcal biofilms: a coordination of physiological signals. *Curr. Opin. Microbiol.* 15, 1–5.
- Sadykov, M. R., Hartmann, T., Mattes, T. A., Hiatt, M., Jann, N. J., Zhu, Y., Ledala, N., Landmann, R., Herrmann, M., Rohde, H., Bischoff, M., and Somerville, G. A. (2011). CcpA coordinates central metabolism and biofilm formation in *Staphylococcus epidermidis*. *Microbiology* 157, 3458–3468.
- Sadykov, M. R., Olson, M. E., Halouska, S., Zhu, Y., Fey, P. D., Powers, R., and Somerville, G. A. (2008). Tricarboxylic acid cycle-dependent regulation of *Staphylococcus epidermidis* polysaccharide intercellular adhesin synthesis. *J. Bacteriol.* 190, 7621–7632.
- Schleif, R. (2010). AraC protein, regulation of the 1-arabinose operon in *Escherichia coli*, and the light switch mechanism of AraC action. *FEMS Microbiol. Rev.* 34, 779–796.
- Seidl, K., Goerke, C., Wolz, C., Mack, D., Berger-Bachi, B., and Bischoff, M. (2008). *Staphylococcus aureus* CcpA affects biofilm formation. *Infect. Immun.* 76, 2044–2050.
- Stoodley, P., Sauer, K., Davies, D. G., and Costerton, J. W. (2002). Biofilms as complex differentiated communities. *Annu. Rev. Microbiol.* 56, 187–209.
- Tamber, S., Schwartzman, J., and Cheung, A. L. (2010). Role of PknB kinase in antibiotic resistance and virulence in community-acquired methicillin-resistant *Staphylococcus aureus* strain USA300. *Infect. Immun.* 78, 3637–3646.
- Tormo, M. A., Marti, M., Valle, J., Manna, A. C., Cheung, A. L., Lasa, I., and Penades, J. R. (2005). SarA is an essential positive regulator of *Staphylococcus epidermidis* biofilm development. *J. Bacteriol.* 187, 2348–2356.
- Tsang, L. H., Cassat, J. E., Shaw, L. N., Beenken, K. E., and Smeltzer, M. S. (2008). Factors contributing to the biofilm-deficient phenotype of *Staphylococcus aureus sarA* mutants. *PLoS One* 3:e3361. doi: 10.1371/journal.pone.0003361
- Tu Quoc, P. H., Genevaux, P., Pajunen, M., Savilahti, H., Georgopoulos, C., Schrenzel, J., and Kelley, W. L. (2007). Isolation and characterization of biofilm formation-defective mutants of *Staphylococcus aureus*. *Infect. Immun.* 75, 1079–1088.
- Ulrich, M., Bastian, M., Cramton, S. E., Ziegler, K., Pragman, A. A., Bragonzi, A., Memmi, G., Wolz, C., Schlievert, P. M., Cheung, A., and Doring, G. (2007). The staphylococcal respiratory response regulator SrrAB induces *ica* gene transcription and polysaccharide intercellular adhesin expression, protecting *Staphylococcus aureus* from neutrophil killing under anaerobic growth conditions. *Mol. Microbiol.* 65, 1276–1287.
- Valle, J., Toledo-Arana, A., Berasain, C., Ghigo, J. M., Amorena, B., Penades, J. R., and Lasa, I. (2003). SarA and not sigmaB is essential for biofilm development by *Staphylococcus aureus*. *Mol. Microbiol.* 48, 1075–1087.
- Vergara-Irigaray, M., Maira-Litran, T., Merino, N., Pier, G. B., Penades, J. R., and Lasa, I. (2008). Wall teichoic acids are dispensable for anchoring the PNAG exopolysaccharide to the *Staphylococcus aureus* cell surface. *Microbiology* 154, 865–877.
- Vuong, C., Kidder, J. B., Jacobson, E. R., Otto, M., Proctor, R. A., and Somerville, G. A. (2005). *Staphylococcus epidermidis* polysaccharide intercellular adhesin production significantly increases during tricarboxylic acid cycle stress. *J. Bacteriol.* 187, 2967–2973.
- Vuong, C., Saenz, H. L., Gotz, F., and Otto, M. (2000). Impact of the *agr* quorum-sensing system on adherence to polystyrene in *Staphylococcus aureus*. *J. Infect. Dis.* 182, 1688–1693.
- Wang, C., Fan, J., Niu, C., Wang, C., Villaruz, A. E., Otto, M., and Gao, Q. (2010). Role of *spx* in biofilm formation of *Staphylococcus epidermidis*. *FEMS Immunol. Med. Microbiol.* 59, 152–160.
- Wang, L., Li, M., Dong, D., Bach, T. H., Sturdevant, D. E., Vuong, C., Otto, M., and Gao, Q. (2008). SarZ is a key regulator of biofilm formation and virulence in *Staphylococcus epidermidis*. *J. Infect. Dis.* 197, 1254–1262.
- Wang, X., Niu, C., Sun, G., Dong, D., Villaruz, A. E., Li, M., Wang, D., Wang, J., Otto, M., and Gao, Q. (2011). *ygs* is a novel gene that influences biofilm formation and the general stress response of *Staphylococcus epidermidis*. *Infect. Immun.* 79, 1007–1015.
- Xu, L., Li, H., Vuong, C., Vadyvaloo, V., Wang, J., Yao, Y., Otto, M., and Gao, Q. (2006). Role of the *luxS* quorum-sensing system in biofilm formation and virulence of *Staphylococcus epidermidis*. *Infect. Immun.* 74, 488–496.
- Ziebuhr, W., Krimmer, V., Rachid, S., Lossner, I., Gotz, F., and Hacker, J. (1999). A novel mechanism of phase variation of virulence in *Staphylococcus epidermidis*: evidence for control of the polysaccharide intercellular adhesin synthesis by alternating insertion and excision of the insertion sequence element IS256. *Mol. Microbiol.* 32, 345–356.

**Conflict of Interest Statement:** The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Received: 09 January 2012; paper pending published: 06 February 2012; accepted: 05 March 2012; published online: 26 March 2012.

Citation: Cue D, Lei MG and Lee CY (2012) Genetic regulation of the intercellular adhesion locus in staphylococci. *Front. Cell. Inf. Microbiol.* 2:38. doi: 10.3389/fcimb.2012.00038

Copyright © 2012 Cue, Lei and Lee. This is an open-access article distributed under the terms of the Creative Commons Attribution Non Commercial License, which permits non-commercial use, distribution, and reproduction in other forums, provided the original authors and source are credited.