Review
Future Microbiology

# *Staphylococcus aureus* adaptation to the host and persistence: role of loss of capsular polysaccharide expression

Lorena Tuchscherr[1], Bettina Löffler[1], Fernanda R Buzzola[2,3] & Daniel O Sordelli[†2,3]
[1]Institut für Medizinische Mikrobiologie, Universitätsklinikum Münster, Germany
[2]Universidad de Buenos Aires, Paraguay, Buenos Aires, Argentina
[3]Consejo Nacional de Investigaciones, Científicas y Técnicas (CONICET), Buenos Aires, Argentina
[†]Author for correspondence: Departamento de Microbiología, Facultad de Medicina, Universidad de Buenos Aires, Paraguay 2155 P-12, (C1121ABG) Buenos Aires, Argentina ■ Tel.: +54 115 950 9618 ■ Fax: +54 114 964 2554 ■ sordelli@fmed.uba.ar

A vast array of virulence factors enable *Staphylococcus aureus* to readily adapt to different environmental niches in diverse hosts. The *cap* gene cluster is present in almost all relevant clinical *S. aureus* isolates and capsular polysaccharide expression is apparent in isolates from patients with acute infection. The number of *S. aureus* isolates from patients with chronic infections that do not express capsular polysaccharide, however, is significantly high, indicating that loss of capsular polysaccharide expression may be a key *S. aureus* feature associated with persistence. The role of the loss of capsular polysaccharide expression as well as the emergence of other defined phenotypes and their relevance to persistence of *S. aureus* and chronicity of the infection is discussed in this article.

*Staphylococcus aureus* is an opportunistic pathogen that can infect, replicate and persist in humans and domestic animals of economic importance [1] and it is a worldwide threat to public health and a liability to the dairy industry [2]. Although *S. aureus* may colonize mucosal surfaces of normal humans with unnoticeable or mild clinical features, it can cause skin and soft-tissue infections, and it has the invasive potential to generate life-threatening infections, including osteomyelitis, endocarditis and bacteremia with metastatic complications [1]. The control of these *S. aureus* infections has been deeply hampered by isolation of methicillin-resistant *S. aureus* from patients with nosocomial and community-acquired infections [3,4]. The situation promises to become even worse by the increasing prevalence of clinically relevant isolates with reduced susceptibility to vancomycin [5] and by the appearance of methicillin-resistant *S. aureus* resistant to vancomycin [6]. However, the emergence of antibiotic-resistant bacterial strains only to some extent explains the existence of persistent and difficult-to-eradicate infections, since many relapsing and therapy-refractory infections are caused by strains that have been found to be susceptible to antibiotics *in vitro*. Currently, the mechanism(s) for persistence in the presence of host defenses and antibiotic therapy are not fully understood.

The *S. aureus* genome carries a vast array of genes coding for virulence and evasion factors. The genes that code for these many factors are conserved in the *S. aureus* genome and display broad functionality with considerable redundancy [1]. The capacity of this microorganism to cause a wide variety of diseases partially depends on its ability to express a number of these factors that, acting in concert, confer resistance to innate and acquired immune defense mechanisms and permit adaptation of the pathogen to distinct and changing environmental niches during infection. The genetic background of *S. aureus* isolates influences the development of disease in human [7,8] and animal hosts [9,10]. *S. aureus* strains differ in virulence potential, but the genetic basis for these differences may not be reflected by multilocus sequence typing [11]. Beyond the fact that *S. aureus* with certain virulence features may be selected in nature to cause certain specific diseases, the evolution of *S. aureus* within affected tissue appears to play a key role in persistence of the microorganism and chronicity of the infection [12]. There is undisputable evidence showing that certain diseases caused by *S. aureus*, such as osteomyelitis, start as acute and later develop into chronic infection [13]. As the acute infection progresses, sections of dead bone tissue are formed that can later detach to create separate infectious foci due to loss of vasculature and lead to persistence of *S. aureus* and chronic infection [14,15]. Therefore *S. aureus* adapts to the different microenvironments in the infection and adjacent sites and selection pressure exerted by host factors may determine the

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emergence of mutants and/or different phenotypes better adapted to the evolving conditions at these infection sites. The high plasticity of the *Staphylococcus* genome makes this genus highly adaptive to environmental changes, which lead to significant phenotypic diversification of *S. aureus* clinical isolates [16]. Whereas *S. aureus* organisms isolated from several specimens taken from single patients with acute infection display an identical genotype and phenotype, similar specimens taken from patients with chronic infections exhibited diverse genetic subtypes and dissimilar phenotypes [12]. Furthermore *S. aureus* recovered from individual patients with chronic disease revealed a heterogeneous population of staphylococci [17]. One virulence factor that was addressed in recent studies concerning chronicity of *S. aureus* infections is the capsular polysaccharide (CP). In this article, the role of CP in chronic *S. aureus* infection is addressed.

## CP expression & disease

Although the existence of up to 11 *S. aureus* CP serotypes has been suggested, only the CPs from serotypes 1, 2, 5 and 8 (CP1, CP2, CP5 and CP8, respectively) have been purified and chemically characterized [18]. The proteins responsible for CP1 synthesis and expression are coded by a 15-gene (*capA* to *capO*) cluster. This cluster is located within a staphylococcal cassette chromosomal (SCC) element similar to the type III SCC*mec* associated with methicillin resistance in *S. aureus* and positioned at the same site as all the SCC*mec* elements [19]. CP-1 and -2 are extremely rare and are very seldom isolated from clinically relevant human clinical specimens. By contrast, CP5 and CP8 are produced by a significant proportion of human *S. aureus* isolates [18]. The genes responsible for CP5 and CP8 biosynthesis are chromosomal and allelic. The CP5(8) locus contains 16 closely linked genes (*cap5(8)A–cap5(8)P*) transcribed in one orientation. Both CP5 and CP8 are composed of the same three sugar residues, ManNAcA, l-FucNAc and d-FucNAc and, therefore, 12 of the 16 genes in the two gene clusters are almost identical. Conversely, four open reading frames located in the central region (*cap5(8)HIJK*) exhibit little homology to each other and determine the CP type-specificity [18]. Current evidence supports the existence of no CP serotypes other than 1, 2, 5 and 8 in *S. aureus* [20]. On an exceptional note, segregation of variants lacking CP expression has been seen in a defined geographical region. Indeed, a prevalent *S. aureus* clone in bovines of Argentina with subclinical mastitis exhibited a deletion of almost the entire *cap* cluster. The deletion was associated with the presence of an insertion element, IS*cap* [21]. The fact that the 63 bp of the 3′ end of the *capP* gene remained in place confirmed that although the *cap* gene cluster was deleted, it was initially present in the genome. It is important to note that subclinical mastitis is a chronic condition involving long-term inhabitancy of the bacteria in the infected udder. Overall, these data allow the conclusion that the *cap* gene cluster (*cap5*[*8*] alleles) is extremely conserved in the *S. aureus* genome.

Whereas the *cap5(8)* genes are conserved in the *S. aureus* genome not all clinically relevant *S. aureus* isolates from humans produce CP5 or CP8 [12,21]. Furthermore, loss of CP5(8) expression has been associated with persistence of *S. aureus* in the infected host. This hypothesis has been supported by different experimental and clinical studies. Studies in a mouse model of mastitis have shown that an isogenic mutant lacking CP expression persisted in higher numbers and for a longer time in the mammary glands compared with their capsulated counterparts [22]. A field study involving bovines revealed a very high proportion (86%) of nontypeable (NT) *S. aureus* in cows with subclinical mastitis [23]. Strains that do not react with antibodies to serotypes 5 or 8 and that do not produce mucoid ($CP1^+$ and $CP2^+$) colonies on solid media are referred to as NT. Clinical studies involving humans showed a significantly higher proportion of NT *S. aureus* in patients with chronic osteomyelitis compared with those with acute osteomyelitis [12]. Conversely, in the latter study, *S. aureus* isolates were recovered from the blood of patients with acute disease different from osteomyelitis and 100% of these isolates expressed CP5 or CP8 microcapsules. In both studies the NT variants isolated from chronically infected hosts were stable and conserved their phenotype over successive passages on artificial media, without reversion to the encapsulated phenotype. A variety of mechanisms can explain loss of CP expression, including mutation in any of the *cap* genes essential for CP production, regulatory genes or the promoter region [20]. Deletion, as described above, has only been seen in *S. aureus* of bovine origin of a well-defined geographical region. It is apparent that the NT variants of *S. aureus* may have an advantage over the capsulated wild-type to persist in the chronically infected host. One of the putative advantages may relate to the increased ability of the NT variants to become intracellular within epithelial cells.

*Staphylococcus aureus's* capacity for invasion depends upon the array of factors determined by the genetic background of the bacteria, since the extent of invasiveness relates to certain *spa* types [24]. The different mechanisms and factors involved in *S. aureus* host cell invasion have been reviewed by Sinha and Hermann [25] and recently updated by Sinha and Fraunholtz [26]. From the evolutionary viewpoint, the population of *S. aureus* growing at an infection site originates from a small size inoculum. At a site that offers homogeneous conditions (e.g., pH, redox potential, nutrient concentration, among many others), for example, the bloodstream, it is expected that the growing population of *S. aureus* would display a homogeneous phenotype fully adapted to these conditions. In all other infections sites staphylococci would encounter gradients of microenvironmental conditions. Adaptation to these changing microenvironments would determine the emergence of distinct phenotypes at the infection site. The diverse microenvironmental conditions that *S. aureus* encounters in a host are much more complex than the fixed experimental conditions under which individual mechanisms of cell invasion were investigated. The combination of antibiotics and host factors related to innate and adaptive immune responses to bacteria at the infection site inactivate large numbers of microorganisms thus creating bottlenecks. As a result of those bottlenecks staphylococci with switched phenotypes will be selected through a process that may be thought of as bet hedging [27]. In this regard, bet hedging can be understood as the capacity for high frequency generation of staphylococci with a phenotype better adapted to persistence in the emerging microenvironments rather than a phenotype that replicates faster. During acute *S. aureus* infection these phenotypes would emerge as the result of regulation of the expression of certain virulence factors, such as CP5(8). In the long run, repeated bottlenecks at the infection site can drive the fixation of mutations, which explains the high frequency of *S. aureus* that do not express CP5(8) in hosts (human and animal) with chronic infection [12,23].

## *S. aureus* access to the intracellular milieu

For many years *S. aureus* was defined as an extracellular pathogen, but increasing evidence indicates that *S. aureus* is a facultative intracellular pathogen as well [28,29]. To enter the intracellular environment, bacteria have evolved different strategies, such as expression of adhesins and downregulation of different regulators and virulence factors, for example, CP5(8) microcapsules [30]. Recent work has demonstrated that *S. aureus* is able to invade professional as well as nonprofessional phagocytes, including endothelial and epithelial cells, and osteoblasts [26]. Adhesion to host cells is an essential step for the invasion process and it is mainly mediated by a number of specific staphylococcal surface proteins (adhesins) [25]. Host cell invasion does not require any further active bacterial processes, as live and fixed bacteria are equally taken into the intracellular milieu [31]. *S. aureus* can express multiple adhesins, including cell-wall-anchored or secreted proteins that bind to various host structures with overlapping functions. In addition to proteins, microbial cell wall components, such as teichoic acid polymers, can also play a role in the adhesion process, which is the prerequisite for colonization and host cell invasion [32].

Previous studies have demonstrated that the expression of not only type 1 or 2 capsules, but also the type 5 microcapsule of *S. aureus* interferes with bacterial adhesion by masking adhesins [33–35]. These findings were further supported by a study from Pöhlmann-Dietze *et al.* showing that:

- Adherence is negatively correlated with CP expression;
- Only nonencapsulated bacterial cells are adherent;
- The CP5-negative isogenic *cap5O* mutant displays significantly greater adherence to endothelial cells than the parental strain [36].

Furthermore, Risley *et al.* revealed that CP expression inhibits *S. aureus* clumping factor A (ClfA)-mediated binding to fibrinogen and platelets [37]. This evidence strongly supports that microcapsules can mask adhesins and thereby interfere with the adhesion process. Consequently, loss of CP expression promotes the exposition of adhesins and access of staphylococci to the intracellular milieu.

The adhesion–ligand interaction leads to internalization of the staphylococci thus enabling the microorganisms to find a niche where they can hide and evade defense mechanisms of the host and antimicrobial agents. CP-expressing bacterial cells would most likely survive in the bloodstream, whereas NT variants would adhere, invade and selectively persist within infected tissues. The emergence of *S. aureus* stable variants not expressing CP5(8) would require an element

of selective pressure. It has recently been shown that antibodies to *S. aureus* CP5(8) are able to select such variants during the course of infection in a mouse model of mastitis under intense passive immunization [38]. It was suggested that antibodies to CP5(8) may favor the clearance of encapsulated *S. aureus* from an infected host, but at the same time select for a bacterial subpopulation (NT) that can be internalized within epithelial cells, thereby avoiding further immune clearance. This hypothesis is supported by the observation that in patients with acute systemic infection only encapsulated *S. aureus* expressing CP5(8) are found, whereas a variable proportion of *S. aureus* not expressing CP5(8) are found in hosts with chronic infection [12].

## Intracellular aggression versus adaptation & persistence

Bacterial uptake is an active process of the host cell that involves changes in the host cytoskeleton, resulting in enclosure of bacteria within phagosomes inside the mammalian cells [39]. In the intracellular milieu *S. aureus* has to cope with bacterial degradation mechanisms, which are also present in nonprofessional phagocytes (e.g., autophagy). Certain defined staphylococcal factors, such as the pore-forming α-hemolysin (α-toxin) can activate the autophagic pathway in the host cell [40]. *S. aureus* has also evolved several strategies to deal with the hostile intracellular conditions. On the one hand, *S. aureus* can release a multitude of extracellular products with aggressive potential, including α-toxin and proteases, that can cause inflammatory, pro-apoptotic and cytotoxic effects [39,41,42]. These factors enable the bacteria to destroy host tissue and invade deeper tissue structures. Here, α-toxin plays a major role, whereas other staphylococcal factors apparently contribute to the aggressive potential (Figure 1) [41,43].

On the other hand, *S. aureus* not only has the ability to damage host tissue, but can also persist within different types of host cells (e.g., in endothelial cells or osteoblasts) for long time periods. *S. aureus* can even persist in professional phagocytes, such as macrophages [44,45]. Bacterial long-term persistence is the most likely cause for chronic and therapy-refractory infections. However, to survive intracellularly *S. aureus* needs to avoid inflammatory and immune reactions of the host. To remain as unnoticed as possible, it is suitable for *S. aureus* to downregulate the expression of virulence factors, for example, α-toxin (see below). This could be achieved by *agr* downregulation (Figure 1) [42,46].

*Staphylococcus aureus* can adapt to different environmental conditions in many ways. In previous studies, *S. aureus* long-term persistence has been largely associated with the formation of small colony variants (SCVs), an alteration in the staphylococcal phenotype resulting in a slow growth rate and diminished expression of virulence factors. *In vitro* studies using host cells revealed that SCVs phenotypes can be described as low virulence, but are particularly adapted to the intracellular environment for long-term persistence [46,47]. Because SCVs form a population that grows very slowly, Proctor *et al.* have hypothesized that SCV populations are not able to reach quorum sensing conditions to activate the *agr* system [48]. Furthermore, Goerke *et al.* have found that CPs are not expressed and the *agr* system was inactive in SCVs isolated from the lungs of cystic fibrosis patients [49].

Loss of CP5(8) expression by *S. aureus* appears to play a role in the emergence of SCVs during the course of infection. Studies in the mouse model of mastitis under intense passive immunization have shown that the presence of antibodies to CP5(8) promotes the emergence of not only NT *S. aureus* but also SCVs. Whether the emergence of SCVs is the direct consequence of previous internalization of *S. aureus* NT variants or a phenomenon independent of CP5(8) expression remains obscure and merits further investigation. Stable SCVs emerging in mice with experimental mastitis under passive immunization with anti-CP5(8) antisera express measurable levels of CP5(8) [38]. Therefore, permanent loss of CP5(8) expression does not appear to be required for SCV emergence. *S. aureus*, however, can downregulate expression of CP5(8) during infection and transiently display a NT phenotype that can be more efficiently internalized owing to uncoated adhesions (Figure 2).

## Regulation of *S. aureus* CP expression

There is no doubt that CP5 and CP8 play a role in the pathogenesis of *S. aureus* infection [50] and, for this reason, these CPs have been repeatedly identified as vaccine candidates [51]. Expression of CP5(8) appears to be essential for dissemination of *S. aureus* from the primary infection site and contributes to the invasive capacity of *S. aureus*. This statement apparently contradicts the findings of high proportions of NT *S. aureus* in certain staphylococcal infections. In addition to the fact that stable NT variants emerge during chronic infection it is important to note that *S. aureus* has an exquisite regulatory network that enable the bacteria

to switch on or off expression of virulence and evasion factors in response to changing environmental conditions. This ability may be the key to the pathogenesis of chronic infection because it would permit adaptation of *S. aureus* to changing microenvironments during the course of infection and the survival and persistence of the bacteria. But before selection of variants with stable NT phenotypes may take place as endpoints of this short-sighted evolution, downregulation of CP5(8) expression may permit the emergence of unstable NT variants that would favor persistence and chronic infection.

Coordinated expression of *S. aureus* virulence factors appears to be critical to the evolution of infection. *S. aureus* can change its lifestyle between 'adherent' and 'aggressive' in response to bacterial density sensed by the *agr* quorum-sensing system [52]. This evidence suggests that *agr* inactivation can be advantageous to *S. aureus* for intracellular survival. When the *agr* is autoinduced, it stimulates the production of exoproteins and inhibits the production of cell-surface proteins during the late logarithmic phase of bacterial growth. Indeed, an increase in the capacity for cell invasion occurs after inhibition of the *S. aureus agr* system wherein the expression of cell wall proteins is elevated [53]. In addition to the ability to sense the cell population density, *S. aureus* can alter the expression of certain genes in response to different signals from the environment surrounding the microorganism. This adaptation often involves a two-component system, consisting of a sensor (histidine kinase) and a response regulator, which are activated by phosphorylation. In *S. aureus* 16-putative two-component regulatory systems that are able to respond to environmental signals have been identified. The *saeRS* system belongs in this category and it was found to be essential for *in vivo* expression of virulence genes [54,55]. The *sae* system exhibits a complex transcriptional pattern strongly influenced by environmental factors [55–57]. The synthesis of extracellular proteins (encoded by *hla*, *hlb*, *coa*, *sspA*, *spa*, *eap*, *emb* and *fnbA*) is positively regulated by *saeRS* and the expression of the *cap* operon is repressed by *saeRS* at the transcriptional level [58]. Furthermore, *S. aureus* has multiple transcriptional factors that directly bind the promoter region of the target genes. In this regard, the best characterized ones are *sarA*, its homologues (*sarR*, *S*, *T*, *U*, *V*, *X*, *Z*, *rot* and *mgrA*) and the alternative factor $\sigma^B$ [59,60]. The transcriptional factor MgrA affects the expression of multiple genes involved in virulence (*cap*, *hla*, *spa*, *nuc*, *sspA*, *coa*) and antibiotic resistance (*norA*, *norB*, *norC*) [61,62].

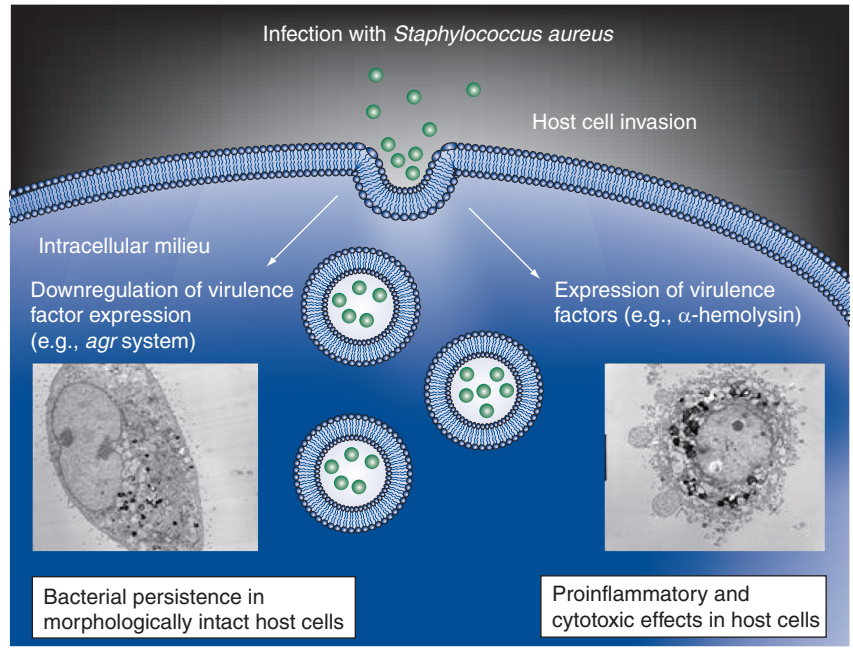


**Figure 1. The course of *Staphylococcus aureus* infection largely depends on host cell invasion and bacterial virulence factor expression.** Downregulation of virulence factor expression establishes a trend towards persistence and chronic infection, whereas upregulation of virulence factor expression contributes to cell damage and acute infection. Photomicrographs previously shown in [39].

The *S. aureus cap5(8)* locus is under the control of a complex regulatory network [18]. The transcription of the *cap* operon in *S. aureus* appears to be controlled by a variety of regulatory elements, such as *yabJ-spoVG, arlRS, agr, sbcDC, ccpA, mgrA, saeRS, sarA* and *KdpDE* [63–67]. CP5(8) expression is stimulated by activation of the *agr* system, the *arlRS* system through *mgrA* [64,68], factor $\sigma^B$ (*sigB*) [65] and *sarA*. Likewise, the *sae* locus has been shown to repress the *cap*5 genes [54]. By contrast, Rogasch *et al.* did not find any influence of SaeRS on the transcription of the *cap* operon [58]. Recently, Zhao *et al.* demonstrated the involvement of the *LuxS*/*AI-2* system in the transcriptional downregulation of the *cap* gene expression in *S. aureus* via a signaling process that involves the two-component system *kdpDE* [67].

Successful adaptation of the pathogen to the human host is achieved by regulatory mechanisms in the short term and by heritable shifts in the population over the long term. *In vivo* expression of CP5(8) was demonstrated a number of years ago in a mouse model of nasal colonization [69], in different animal models of acute infection, for example, endocarditis [70,71] and subcutaneous infection [72], and in cows with mastitis [73]. By contrast, CP5 expression was not detected *in vivo* in other staphylococcal infections. In rats challenged with a CP5$^+$ *S. aureus* strain

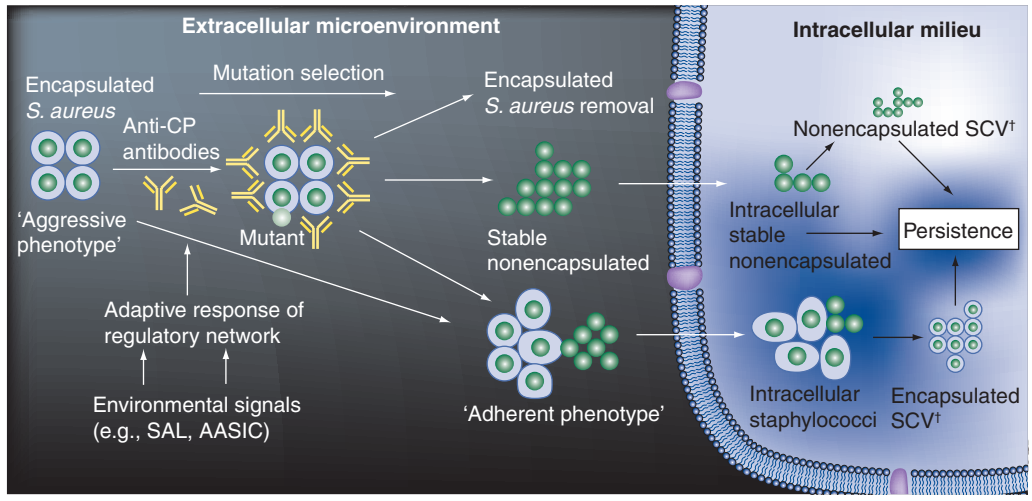


**Figure 2. The *Staphylococcus* microevolution from 'aggressive' to 'adherent' phenotype hypothesis *in vivo*.** Antibodies to CP5(8) opsonize capsulated *S. aureus* ('aggressive phenotype') and lead to its subsequent removal by professional phagocytes. At the expected rate for a point mutation, stable mutants that do not express CP5(8) (nontypeable [NT] variants) emerge and are selected ('adhesive phenotype'). If enough time elapses, total selection of a NT, stable *S. aureus* occurs in the chronically infected host. These NT staphylococci are more efficiently internalized. If loss of CP5(8) occurs owing to a mutation in a regulatory system, concomitant loss of other factors such as α-hemolysin also occurs, and such *S. aureus* variants are better adapted to the intracellular lifestyle. NT variants would precede the emergence of nonencapsulated SCVs (from left to right, upper portion of the figure). But before a mutation is fixed and stable NT variants are selected, within a more reduced timeframe (acute infection), regulation of virulence factor expression occurs. Expression of *S. aureus* regulators may be modulated by certain molecules at the infection site, such as salicylic acid, antibacterial agents in subinhibitory concentration and/or components of the innate immune system, also leading to the emergence of NT *S. aureus*. These *S. aureus* will gain access to the intracellular milieu, where they may evolve into capsulated SCVs. *S. aureus* SCVs with both capsulated and noncapsulated (NT) phenotype can be found *in vivo*. The SCV is a phenotype extremely well adapted to the intracellular lifestyle (from left to right, lower portion of the figure). The emergence of NT variants that also fail to produce other virulence factors and the SCVs permit persistence of *S. aureus* in the chronically infected host.
†The figure is extremely simplified and does not include the adhesins and receptors involved in internalization or other events that occur in the intracellular milieu. The diagram is not to scale; for instance staphylococci that produce SCV colonies on solid culture media are actually larger in size than the wild-type parental *S. aureus* microorganisms.
AASIC: Antibacterial agents in subinhibitory concentration; CP: Capsular polysaccharide; SAL: Salicylic acid; SCV: Small colony variant.

in the granuloma pouch model, less than 5% of the cells collected from the pouch exudates were CP5$^+$ [74]. Similarly, minimal expression of CP5 was observed in either lung tissue or nasal polyp tissue obtained from two cystic fibrosis patients infected with *S. aureus* [35,74]. The absence of CP5 expression correlated with elevated $CO_2$ levels (≥4%) in both cases. Goerke and Wölz investigated *S. aureus* recovered from cystic fibrosis patients [16] and more recently further expanded their research to show that *S. aureus* has evolved a variety of strategies to adapt to the cystic fibrosis lung [49]. These include the emergence of isolates with mutations in metabolic (e.g., SCVs) and regulatory (e.g., *agr* mutants) genes. But *S. aureus* can also adapt to the cystic fibrosis lung using regulatory mechanisms that are not well defined in the context of this disease. The quorum-sensing *agr* system is not activated during lung infection in cystic fibrosis, which is consistent with a proposed biofilm mode of growth in the lungs and also with the observation that in cystic fibrosis patients *S. aureus* does not usually disseminate to cause systemic disease. Adaptation of *S. aureus* to the cystic fibrosis lung therefore leads to the generation of a phenotypically heterogeneous *S. aureus* population that expresses factors required for persistence rather than virulence.

CP5(8) production occurs mainly during the postexponential phase of growth when glucose is growth limiting and tricarboxylic acid cycle intermediates are required [75]. Under carbohydrate-rich conditions, the repressed synthesis of CP5(8) is mediated, at least in

part, by catabolite control protein A (CcpA) [66]. However, no catabolite responsive elements (*cre*)-site was identified near the *cap* genes, suggesting that CcpA affected *cap* transcription indirectly [66]. On the other hand, the repressor CodY (a regulator involved in nitrogen metabolism) [76] downregulated the *cap* transcripts in *S. aureus* by direct repression and by repression of the *agr* locus [77,78]. In addition, the expression of CP5(8) in *S. aureus* is highly sensitive to several environmental signals, such as high salt concentration and pH [18]. Therefore, it is expected that CP5(8) expression may be influenced by the conditions that *S. aureus* encounters in different *in vivo* microenvironments.

Certain commonly used pharmaceutical agents affect the expression of regulatory systems of *S. aureus* and virulence factors under their control [79–81]. It has been shown that under a subinhibitory concentration of ciprofloxacin, the transcription of *cap* was reduced owing to the repressive action of *sbdDC* through an *arl-mgr* pathway [63]. Recent results from our laboratory demonstrated that the exposure of encapsulated *S. aureus* strains to low concentrations of salicylic acid (the main aspirin biometabolite) increased the ability of the bacteria to invade epithelial cells [82]. Interestingly, this increased invasive ability correlated with a diminished production of CP5(8). Moreover, salicylic acid treatment of *S. aureus* reduced *cap* expression at the transcriptional level, as well as the activity of the major *cap* promoter [82]. In addition, diminished transcription of *mgrA* and upregulation of the *saeRS* transcripts were found. Collectively, the experimental evidence suggests that *S. aureus* is able to reduce CP5(8) expression in response to defined environmental signals (e.g., pharmacological agents, nutritional conditions) which are detected by the regulatory network, thus promoting the emergence of an 'adherent phenotype', whose surface proteins are entirely exposed. It can be speculated that the *S. aureus* regulatory systems can react to certain environmental conditions and adapt for persistence, thus modifying the progression of infection towards chronicity.

## Future perspective

The transition between acute and chronic infection by *S. aureus* is a process that remains obscure. How the first staphylococci that gain access to the host evolve into the end-point phenotypes found in many chronically infected patients remains to be elucidated. We speculate that *S. aureus* regulates the features of the microorganisms by a complex but fast-reacting system to make possible to shift from a colonization status to an acute infection condition and further down the microevolution to eventually lead to a persistent or chronic infection state. Many factors appear to be involved in this process and *S. aureus* CP5(8) is undoubtedly one of these. One important finding to deem relevant is the increased prevalence of NT *S. aureus* isolates recovered very frequently from humans suffering from chronic infection, especially since in these patients chronic infection is refractory to antimicrobial therapy. According to the stage of host-bacteria interaction, *S. aureus* may upregulate CP5(8) expression in order to avoid the immune response effectors during the bloodstream lifestyle and further downregulate it to permit internalization into a target cell at the tissue to be metastasized. Such internalization would be mediated by uncoated surface adhesins and *S. aureus* would then persist in the intracellular milieu of epithelial cells where they are protected from competent phagocytes and host immune system strategies. The transition from

## Executive summary

- *Staphylococcus aureus* isolated from multiple specimens of patients with chronic infection reveal diversity, such as the presence of diverse genetic subtypes and dissimilar phenotypes, including nontypeable (NT)-stable variants.
- The nonencapsulated variants of *S. aureus* may have an advantage over the capsulated ones to persist in the chronically infected host. One of the putative advantages is the increased ability of NT variants to become intracellular within epithelial cells wherein they avoid further immune clearance.
- During infection, the presence of antibodies to *S. aureus* capsular polysaccharide serotypes 5 and 8 (CP5[8]) is one of the host factors that select for NT variants, which can more efficiently enter the intracellular milieu.
- Permanent loss of CP5(8) expression during infection does not appear to be required for small colony variant emergence.
- Emergence of regulatory (unstable) *S. aureus* NT variants during infection before stable mutations are fixed may play a key role in the early events leading to internalization of *S. aureus* and ultimate persistence.

an extracellular to an intracellular life deserves further research to understand which factors are being switched on and off at that point. The comprehension of these steps will provide candidate molecules to design better therapeutic approaches and to add to the composition of an effective oligocomponent vaccine.

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- ▪ of interest
- ▪▪ of considerable interest

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