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The control of death and lysis in staphylococcal biofilms: a coordination of physiological signals

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Abstract

The processes involved in the development of complex multicellular communities, including the programmed elimination of individual cells during the formation of specialized structures, exhibits fundamental similarities between prokaryotic and eukaryotic organisms. Mechanistic similarities may also exist at the molecular level, as bacterial proteins hypothesized to be related to the apoptosis regulator Bax/Bcl-2 family, have been identified, fueling speculation about the existence of bacterial PCD. Here we review the regulatory networks controlling cell death and lysis in *Staphylococcus aureus* and examine the environmental parameters that might influence them during the development of a biofilm. We hypothesize that the heterogeneous environmental conditions found within a developing biofilm generate distinct physiological signals that coordinate the differential expression of cell death and lysis effectors.

Introduction

Bacterial programmed cell death (PCD) has been hypothesized to be involved in sporulation, genetic exchange, elimination of damaged and defective cells, control of viral infection, and limiting the rate of spontaneous mutations that have the potential to take over the population [1,2••,3]. PCD has also been proposed to function in many respects like it does in more complex organisms, in the development of multicellular structures, for example, in fruiting body formation by *Myxococcus* sp. [4–6]. Similarly, cell death and lysis within a biofilm population is well documented [7–12•], in many cases effecting biofilm architecture and leading to the release of cytoplasmic contents including genomic DNA [13,14]. This extracellular DNA (eDNA) can contribute to antibiotic resistance [15], promote horizontal gene transfer [16] and, by virtue of its adhesive properties, play an essential role in the biofilm matrix as an adherence molecule [14,17,18•,19].

The *Staphylococcus aureus* Cid/Lrg system

In *Staphylococcus aureus*, our understanding of molecular mechanisms controlling death and lysis during biofilm development is centered largely on the *cidABC* and *lrgAB* operons [12,14]. It was proposed that these operons may have an important role in the control of staphylococcal murein hydrolase activity and encode proteins analogous to the

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bacteriophage-encoded holins and antiholins [20,21]. Holins and antiholins are members of a large family of membrane proteins that are directly involved in regulation of bacteriophage-induced death and lysis. Specifically, they control the activity of bacteriophage-encoded murein hydrolases (i.e. endolysins) and the timing of lysis during bacteriophage infection by regulating the access of these enzymes to their substrate, peptidoglycan [22••]. This regulation can be achieved by one of two proposed mechanisms: through control of murein hydrolase transport across the cytoplasmic membrane or by mediating the release and activation of membrane-associated murein hydrolases [23–26]. Antiholins are homologous proteins (in fact, through a "dual start motif" they are commonly encoded in bacteriophage by the same gene encoding the holin) that have an inhibitory effect on holin activity [27]. Similarly opposing activities in controlling cell lysis and genomic DNA release during biofilm development have also been observed for the *cid* and *lrg* gene products, suggesting that *cid* encodes a holin and *lrg* encodes an antiholin [28] (see Figure 1). Additional support for holin-like properties of *cid* and *lrg* gene products was recently obtained by biochemical and molecular characterization of CidA and LrgA proteins that demonstrated the oligomerization and association of both CidA and LrgA with the cytoplasmic membrane [29]. Importantly, mutations in either the *cid* or *lrg* operon result in altered biofilm formation [12,14] suggesting that a careful balance between life and death is critical for biofilm development, not unlike development in more complex eukaryotic systems [30]. Given the similarities of holins and antiholins to the Bax/Bcl-2 family of proteins, it has been postulated that these seemingly unlinked proteins are also functionally related [30,31]. Support for this idea has recently come from the demonstration that Bax could functionally complement a holin deficiency in bacteriophage lambda [32]. Furthermore, the role of a plastid-associated "LrgB family" protein in plant senescence was recently described [33••] suggesting a broader role for these proteins than what was previously appreciated.

Regulation of the *Staphylococcus aureus* Cid/Lrg system

The presence of a system controlling bacterial cell fate is almost certainly going to be associated with a complex regulatory mechanism. Indeed, there are two regulatory networks controlling cell death and lysis mediated by the action of Cid/Lrg system including the LytSR two-component regulatory system and the LysR-type transcriptional regulator known as CidR.

The LytSR two-component regulatory system

Our studies of the *S. aureus cid* and *lrg* operons were initiated over a decade ago, with the identification of the LytSR two-component regulatory system that affects murein hydrolase activity and autolysis [34]. It has been shown that disruption of the *lytSR* genes causes increased autolysis and altered levels of murein hydrolase activity, suggesting that the LytS and LytR proteins are involved in controlling autolysis in *S. aureus* by affecting the intrinsic murein hydrolase activity associated with the cell [34]. The LytSR system consists of a sensor histidine protein kinase (LytS) and a response regulator (LytR) and has been shown to function, in part, by inducing *lrgAB* expression [35]. Current evidence suggests that LytS senses some aspect of membrane potential, since agents that dissipate this component of the proton motive force induce *lrgAB* expression in a *lytSR*-dependent manner [36]. It has also been shown that *lrgAB* expression is strongly induced in a *lytSR*-dependent manner during overflow metabolism (i.e. under conditions of excess of glucose and oxygen) and coincides with accumulation of acetate in the media [37]. Interestingly, recent studies demonstrate that under conditions of overflow metabolism, LytR alone can complement *lytSR* inactivation and induce *lrgAB* transcription (Sharma-Kuinkel and Bayles; unpublished results). These observations could be explained by a model in which a small phosphodonor molecule, such as acetyl phosphate, directly phosphorylates LytR independent of LytS (Figure 1). This is in

agreement with the findings that many other well-characterized response regulators can be activated by small phosphodonor molecules such as acetyl phosphate or carbamoyl phosphate [38–41]. Additional support for this model comes from the fact that during aerobic growth of *S. aureus*, glucose is predominantly catabolized through the Pta-AckA pathway, of which acetyl phosphate is an intermediate [42•]. Thus, the ability of LytR alone to induce *lrgAB* transcription during overflow metabolism may be a consequence of increased acetyl phosphate pools and the direct phosphorylation of LytR by this molecule.

The CidR regulator

The analysis of the *S. aureus* genome sequence revealed an open reading frame located immediately upstream of *cidABC* locus, designated *cidR*, that encodes a putative LysR-type transcriptional regulator (LTTR) [43]. Similar to other members of the LTTR family, the CidR amino acid sequence contains a conserved N-terminal helix-turn-helix motif responsible for DNA binding [44] and a putative C-terminal regulatory domain that is involved in binding of a small co-inducer molecule [44–46]. Inactivation of the *cidR* gene has a negative effect on *cidABC* transcription and results in reduced murein hydrolase activity and survival during stationary phase [43]. Northern blot analysis of *cid* transcription revealed the presence of two overlapping transcripts designated *cidABC* and *cidBC*. Expression of the smaller transcript is dependent on sigma factor B [47], while the full-length *cidABC* transcript is induced by growth in the presence of excess glucose and/or acetic acid and is CidR dependent [37,43]. Furthermore, comparison of the transcriptional profiles of the *cidR* mutant with the parental strain revealed that only two operons, *alsSD* and *cidABC*, were upregulated by CidR [48]. The products of the *alsS* and *alsD* genes are acetolactate synthase and acetolactate decarboxylase, respectively, and like the *cidC*-encoded pyruvate oxidase, these proteins are involved in catabolism of pyruvate formed under excess glucose conditions. However unlike pyruvate oxidase that decarboxylates pyruvate to acetate [49], these enzymes belong to the 2,3-butanediol pathway, converting pyruvate to the neutral byproduct, acetoin [48,50]. Since members of the LTTR family typically require a small co-inducer molecule for activation of their target genes [44], we speculate that pyruvate or an intermediate of pyruvate metabolism might serve as this molecule (Figure 1).

Control of Cid/Lrg expression during biofilm development

Given the nature of the signals known to induce expression of the Cid/Lrg system, we propose that control of *cid* and *lrg* expression depends upon the varied environmental conditions that exist within different micro-niches of a biofilm [51•,51,53•,54,55••,56]. But how might the biofilm micro-environment affect *cid* and *lrg* expression? With respect to *cid* expression, induction in planktonic cultures was observed under excess glucose [37,43], where the high rate of glycolysis inhibits aerobic respiration and induces carbon flow through fermentation pathways, a phenomenon known as the Crabtree effect [57••]. Thus, we considered the possibility that the signal sensed by CidR may be related to fermentative metabolism, which would occur most commonly under hypoxic conditions. Indeed, cell death and lysis are commonly observed in the interior of large biofilm structures, where oxygen levels would presumably be limiting [13,14]. In support of this notion, growth of *S. aureus* under hypoxic conditions resulted in the CidR-dependent induction of *cid* expression (manuscript in preparation). Furthermore, real-time microsensor measurements of local metabolic activities in *ex vivo* dental biofilms show strong acidification of the anoxic layer of biofilm in response to the addition of sucrose [54]. The organic acids responsible for this acidification may also provide signals relevant to LytSR-mediated control of *lrgAB* transcription, as it is well known that weak acids can serve as effective protonophores capable of transporting hydrogen ions through the cytoplasmic membrane barrier and releasing them into the cytoplasm, thus, dissipating the membrane potential [58••,59,60].

Intriguingly, as was mentioned above, CidR function is not limited to the regulation of the *cidA* and *cidB* genes. It also includes regulation of genes involved in diverse pathways of pyruvate catabolism, leading to the generation of acetate and acetoin [48–50]. Hence it is plausible that disruption of the delicate balance in expression of these metabolic genes might also be involved in the control of cell death and lysis [31]. Additionally, DNA microarray analyses revealed that LytSR TCS system in both *S. aureus* and *S. epidermidis* regulates expression of a wide variety of genes involved in major cellular processes like energy, carbohydrate, and nucleotide metabolism [35,61]. These results suggest that LytSR may also affect cell viability and adaptation to an altered environment, not only by preventing cell lysis through up regulation of the synthesis of antiholin molecules, but also by regulating expression of genes controlling the metabolic state of the bacteria.

Concluding remarks

Although we are making progress in understanding the molecular signals involved in the regulation of the genes encoding the Cid/Lrg regulatory system, the mechanisms dictating the differential control of cell death and lysis within a developing biofilm remain poorly understood. Clearly, by virtue of the functional nature of these genes, this cannot be an “all or nothing” phenomenon. Therefore, what processes determine which cells will live and replicate within a biofilm, and which cells will die and lyse, remains an important unanswered question. Although studies of the function and regulation of the *S. aureus* Cid and Lrg proteins have provided a unique perspective on cell death and lysis during biofilm development, we are only at the early stages of appreciating the impact of the biofilm environment on these processes. A more complete understanding of the consequences of these physiological changes and how they contribute to the coordination of metabolic signals leading to cell death and lysis will undoubtedly provide new perspectives on biofilm development.

Highlights

> We discuss the roles of the *Staphylococcus aureus cid* and *lrg* operons in death and lysis during biofilm development. > We describe the function of the *S. aureus* LytSR and CidR regulatory proteins in the control of *cid* and *lrg* expression. > We hypothesize that the metabolic heterogeneity observed within the biofilm environment determines the differential expression of *cid* and *lrg*, which in turn, dictates which cells die and lyse.

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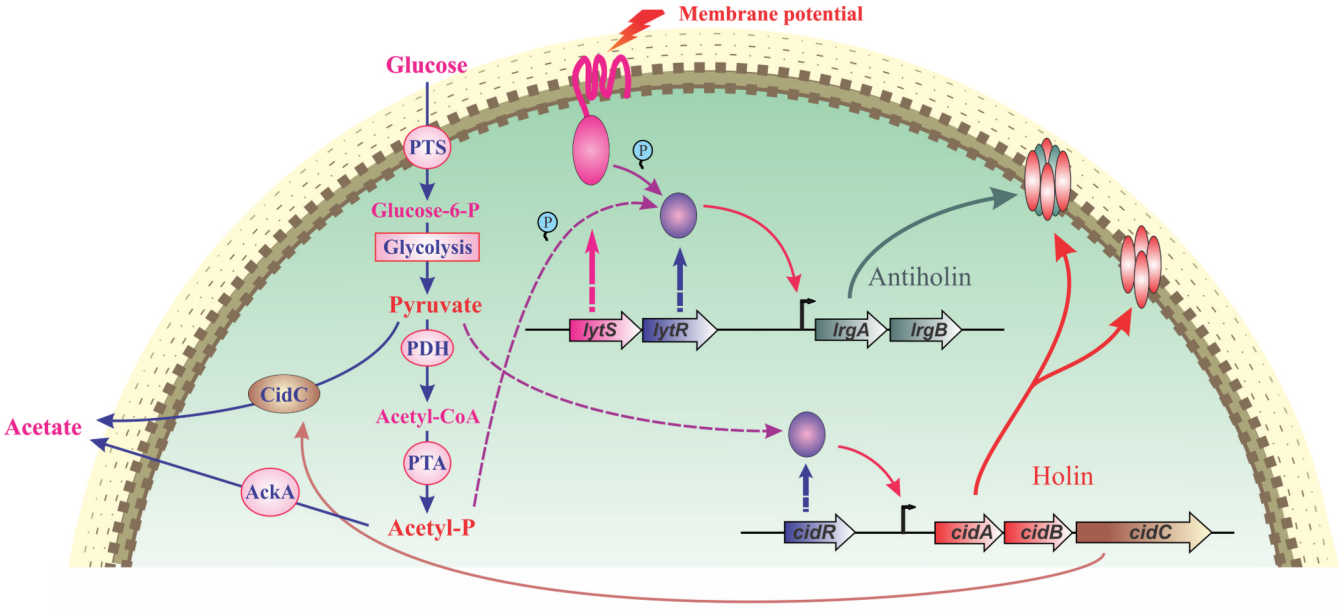

Figure 1. The Cid/Lrg regulatory network

The products of *cidABC* and *lrgAB* operons involved in the control of cell death and lysis in *Staphylococcus aureus*. The *cidA* and *lrgA* genes encode homologous hydrophobic proteins that are hypothesized to function as holins and antiholins, respectively. The *cidB* and *lrgB* genes encode homologous hydrophobic proteins with unknown functions. The *cidC* gene encodes pyruvate oxidase that decarboxylates pyruvate to acetate. The LytSR two-component regulatory system senses decreases in membrane potential and responds by inducing *lrgAB* transcription. The intermediate product of overflow metabolism (shown on the left side) acetyl phosphate (Acetyl-P) is proposed to activate LytR independent of LytS. The LysR-type transcription regulator, CidR, induces *cidABC* transcription during overflow metabolism in response to the accumulation of intracellular pyruvate and/or acetate. Enzymes presented in this scheme that are involved in overflow metabolism in *S. auereus*: PTS, phosphoenolpyruvate-dependent phosphotransferase system; PDH, pyruvate dehydrogenase complex; PTA, phosphotransacetylase; AckA, acetate kinase.