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Multitasking actors of *Staphylococcus aureus* metabolism and virulence

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Abstract (50 words)

Recent studies have uncovered the striking commonality of multitasking molecular actors linking metabolism and virulence regulation. Beyond the well-known importance of carbohydrate and amino acid metabolism regulators in coordinating metabolic pathways and pathogenesis, we highlight recent major advances linking lipid and nucleic acid pathways to virulence regulation in *S. aureus*.

***Staphylococcus aureus* virulence and metabolism**

Staphylococcus aureus, which is both a human commensal and pathogen, is mainly found in the anterior nares, but can also colonize the skin and mucosa. The bacterium is responsible for a wide variety of infections, ranging from mild skin infections to life-threatening bacteremia. When facing different host microenvironments, *S. aureus* must cope with nutrient shifts and modulates its virulence level by well-known interconnected regulatory systems including Agr and SaeRS. Agr system controls the switch from expression of cell-surface associated proteins, including fibronectin-binding proteins (FnBPs; FnbA and FnbB), which are involved in biofilm formation and internalization within host cells, to secreted toxins including alpha-hemolysin. Multiple metabolic factors involved in carbohydrate or amino acid metabolism have been found to moonlight in virulence. We highlight here recent discoveries linking lipid and nucleobase metabolism to *S. aureus* pathogenesis.

Purine and pyrimidine biosynthetic pathways

While investigating the unusual ability of several isolates to form clumps in liquid culture (hyper-clumping phenotype), Goncheva *et al.* identified mutations in the purine biosynthesis repressor gene, *purR* [1]. They discovered that, besides its role in purine pathway regulation, PurR was also involved in virulence modulation. Indeed, *purR* mutants were hypervirulent in a systemic mice infection model. Hypervirulence of the *purR* mutants correlated to their hyper-clumping phenotype in serum, due to increased expression of genes encoding FnBPs. The clinical relevance of *purR* mutations is supported by the fact that *purR* variants are found in public databases of genomes [1]. These variants could emerge during the course of infection since Goncheva *et al.* were able to recover *purR* mutants from the organs of mice initially infected with a wild-type USA300 strain [1].

In a paper published a few months later, Sause *et al.* confirmed that, in a *purR* defective mutant, overexpression of FnbA increases bacterial burden in tissues in a murine model of bacteremia [2]. Sause *et al.* demonstrated direct binding of PurR to the promoters of several virulence genes (including *fnbA*, genes coding for leukocidins and alpha-hemolysin) and regulators, highlighting that multiple factors contribute to the hypervirulence of *purR* mutants independently of purine biosynthesis [2].

One can hypothesize that a decrease in purine availability during the course of infection requires concomitant PurR derepression of both purine biosynthesis pathway and virulence

factor expression. Besides, as shown by Goncheva *et al.* [1], stress induced by the host immune response selects for *purR*-inactivating mutations, which likely confers metabolic advantage that incidentally locks these strains in a hypervirulence state.

A role in pathogenesis has also been recently reported for PyrR, the repressor of the pyrimidine biosynthesis operon, by Copin *et al.* while investigating the epidemic success of a USA300 subclone [3]. In this subclone, a *pyrR*-inactivating mutation increased *pyr* operon gene expression, providing a colonization and transmission advantage in a mouse gastrointestinal model [3].

Collectively, these findings provide novel links between *S. aureus* pathogenesis and nucleobase metabolism through modulation of virulence and colonization potential (Table 1).

The other recent major findings linking pathogenesis and metabolism in *S. aureus* pertain to the regulation of lipid pathways and more specifically highlight the key roles of fatty acids.

Fatty acid (FA) metabolism

FA metabolism has been recently linked to both virulence and colonization potential in *S. aureus*. Fatty acid kinase A (FakA encoded by *fakA* aka *vfrB*) phosphorylates and incorporates exogenous FAs into the *S. aureus* cell membrane [4]. Whereas a *S. aureus* strain lacking FakA exhibits an increased virulence in a mouse model of dermonecrosis [5], it has a virulence defect in a mouse model of bacteremia [6]. While the increased skin virulence remained unexplained, Lopez *et al.* correlated the virulence attenuation observed in the bacteremia model with a lack of type VII secretion system (T7SS) activation. The *S. aureus* T7SS contributes to virulence in murine infection models. The authors suggest that *S. aureus* modulates the expression of its T7SS in response to environmental levels of host-derived FAs. Indeed, the FakA-mediated incorporation of environmental FAs in the bacterial membrane modifies its fluidity, which could trigger T7SS expression [6].

Transcriptional profiling of a *fakA* mutant provided additional explanation for its virulence defect by showing severe attenuation in SaeRS-dependent virulence factor transcription [7]. The SaeRS two-component system is a master regulator of virulence genes in *S. aureus*. Ericson *et al.* demonstrated that FAs that fail to be incorporated into the membrane accumulate within *fakA* mutant cells and have a direct inhibitory effect on SaeRS signaling. Thus, by incorporating FAs into *S. aureus* membranes, FakA modifies both membrane fluidity and the FA intracellular pool, which impact T7SS expression and SaeRS signaling respectively. Interestingly, *fakA* mutations were identified in clinical isolates from cystic

fibrosis patients [8]. Collectively, these studies suggest a key but still underappreciated key role for FakA in *S. aureus* pathogenesis.

Studies on bacterial lipid metabolism may further our understanding of the role of FAs in bacterial pathogenesis, since, in addition of being major energy sources and important component of membranes, lipid-related molecules released by bacteria are signal molecules that can be sensed by other bacteria and by host immune cells. Indeed, bacterial lipoproteins and lipopeptides are pathogen-associated molecular patterns (PAMPs), which are major agonists for Toll-like receptor (TLR) signaling during the innate immune response. By conducting a screen to identify *S. aureus* factors of immune evasion, Grayczyk *et al.* found that a mutation in the lipoic acid synthetase gene (*lipA*) elicited a hyper-inflammatory response in macrophages [9]. Lipoic acid (LA) is a fatty acid-related cofactor covalently linked to multiprotein enzymatic complexes such as pyruvate dehydrogenase (PDH), yielding lipoyl-PDH [9]. Wild type *S. aureus*, but not a *lipA* mutant, releases nanomolar quantities of lipoyl-PDH into the culture supernatant, which is able to blunt TLR2-dependent activation of macrophages. To support the immunosuppressive function of protein lipoylation, Grayczyk *et al.* showed that a synthetic tripeptide containing a lipoyl residue, but not the unmodified tripeptide, was sufficient to suppress TLR1/2-dependent macrophage activation by *S. aureus* lipoproteins [9]. The authors thus proposed that secreted lipoyl-PDH prevents lipoprotein-dependent activation of macrophages through its lipoyl moiety. It is likely that lipoylated proteins antagonize lipoprotein activation of macrophages by competing for TLR binding.

More recently, the predominant *S. aureus* secreted lipase, Geh, was demonstrated to also be a potent immunomodulator [10]. Besides liberating long chain FAs from host-derived lipoproteins for nutritional purpose, Geh also hydrolyzes *S. aureus* lipoproteins, which prevents their recognition by host immune cells and fulfills an elegant immune evasion function, which ultimately promotes persistence [10]. Thus, a protein can exhibit several functions by displaying several activities or by performing the same activity on different targets.

LipA and Geh are two striking examples of *S. aureus* enzymes involved in both lipid metabolism and immune evasion. While LipA seems to prevent lipoprotein activation of TLR, Geh prevents immune cell activation by directly hydrolyzing lipoproteins. Therefore, FakA, LipA and Geh represent multitasking enzymes that play a critical role in *S. aureus* pathogenesis in addition to their function in lipid metabolism (Table 1).

Concluding Remarks

The list of multifunctional proteins frequently referred to as “moonlighting proteins” or multitasking enzymes involved in metabolic pathways and bacterial pathogenesis is rapidly expanding. The online MoonProt Database includes around 400 proteins displaying such moonlighting functions and it is likely that many other moonlighting proteins have yet to be found (<http://www.moonlightingproteins.org/>). Since additional functions can be difficult to predict, multifunctional proteins are often serendipitously discovered during the course of proteomic or genomic data analysis. However, while such omics studies may help uncover unexpected new functions of proteins not previously linked to virulence, the well-known classical virulence factors are resourceful and can still surprise us. In particular, it was known that when located extracellularly, enzymes of glycolysis could contribute to bacterial adhesion. Strikingly, it is now shown that translocation of cytoplasmic proteins into the extracellular milieu by *S. aureus* requires a functional *agr* system through the membrane-damaging activity of phenol-soluble modulins peptides [11].

In addition, *S. aureus* biofilm proteome profiling revealed a high abundance of secreted virulence factors, including hemolysins, leukotoxins, and lipases trapped within the extracellular matrix where they fulfill an additional stabilizing function by generating electrostatic bridges [12]. Altogether, these studies underline how activities of multitasking proteins can be tightly linked with *S. aureus* pathogenicity.

Table 1. Molecular actors of metabolism with a recently described role in *S. aureus* pathogenesis.

| Gene | Metabolic function | Impact on pathogenesis |
|-------------|---|--|
| <i>purR</i> | Repression of purine biosynthesis | Impacts fibronectin binding proteins (FnBPs), leukocidins and α -toxin regulation. <i>purR</i> inactivation leads to hyper-clumping, hypervirulence and increased biofilm formation [1, 2]. |
| <i>pyrR</i> | Repression of pyrimidine biosynthesis | <i>pyrR</i> inactivation provides a colonization and transmission advantage in a mouse gastrointestinal model [3]. |
| <i>fakA</i> | Fatty acid phosphorylation and uptake into <i>S. aureus</i> cell membrane | Impacts type VII secretion system activation [6] and SaeRS-dependent virulence factor transcription [7]. <i>fakA</i> mutants exhibit a virulence defect in a mouse model of bacteremia [6] and an |

| | | |
|-------------|--|--|
| | | increased virulence in a mouse model of dermonecrosis [5]. |
| <i>geh</i> | Hydrolysis of environmental long chain lipids and <i>S. aureus</i> lipoproteins released in the extracellular milieu | By hydrolyzing lipoprotein pathogen-associated molecular patterns (PAMPs), Geh prevents their recognition by host immune cells and allows immune evasion [10]. |
| <i>lipA</i> | Synthesis of lipoic acid (LA), a cofactor that covalently modify proteins such as the pyruvate dehydrogenase (PDH) complex yielding lipoyl-PDH | Lipoyl-proteins antagonize lipoprotein activation of macrophage likely by a competition for TLR binding [9]. |

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