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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*.

23 ***Staphylococcus aureus* virulence and metabolism**

24 *Staphylococcus aureus*, which is both a human commensal and pathogen, is mainly found in
25 the anterior nares, but can also colonize the skin and mucosa. The bacterium is responsible for
26 a wide variety of infections, ranging from mild skin infections to life-threatening bacteremia.
27 When facing different host microenvironments, *S. aureus* must cope with nutrient shifts and
28 modulates its virulence level by well-known interconnected regulatory systems including Agr
29 and SaeRS. Agr system controls the switch from expression of cell-surface associated proteins,
30 including fibronectin-binding proteins (FnBPs; FnbA and FnbB), which are involved in
31 biofilm formation and internalization within host cells, to secreted toxins including alpha-
32 hemolysin.

33 Multiple metabolic factors involved in carbohydrate or amino acid metabolism have been
34 found to moonlight in virulence. We highlight here recent discoveries linking lipid and
35 nucleobase metabolism to *S. aureus* pathogenesis.

36

37 **Purine and pyrimidine biosynthetic pathways**

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

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

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

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

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

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

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

68

69 **Fatty acid (FA) metabolism**

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

81 Transcriptional profiling of a *fakA* mutant provided additional explanation for its virulence
82 defect by showing severe attenuation in SaeRS-dependent virulence factor transcription [7].

83 The SaeRS two-component system is a master regulator of virulence genes in *S. aureus*.
84 Ericson *et al.* demonstrated that FAs that fail to be incorporated into the membrane
85 accumulate within *fakA* mutant cells and have a direct inhibitory effect on SaeRS signaling.
86 Thus, by incorporating FAs into *S. aureus* membranes, FakA modifies both membrane
87 fluidity and the FA intracellular pool, which impact T7SS expression and SaeRS signaling
88 respectively. Interestingly, *fakA* mutations were identified in clinical isolates from cystic

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

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

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

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

121

122 **Concluding Remarks**

123

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

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

142

143

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

146

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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