**Staphylococcus aureus** type I signal peptidase: essential or not essential, that’s the question

Wouter L.W. Hazenbos1,*, Elizabeth Skippington2 and Man-Wah Tan1,*

1 Department of Infectious Diseases, Genentech Inc., 1 DNA Way, South San Francisco, CA 94080, USA.
2 Department of Bioinformatics and Computational Biology, Genentech Inc., 1 DNA Way, South San Francisco, CA 94080, USA.

* Corresponding Authors:
  Wouter Hazenbos, E-mail: whazenbos@yahoo.com
  Man-Wah Tan, E-mail: tan.man-wah@gene.com

Secretion of proteins into the extracellular environment is crucial for the normal physiology and virulence of pathogenic bacteria. Type I signal peptidase (SPase I) mediates the final step of bacterial secretion, by cleaving proteins at their signal peptide once they are translocated by the Sec or twin-arginine (Tat) translocon. SPase I has long been thought to be essential for viability in multiple bacterial pathogens. Challenging this view, we and others have recently created *Staphylococcus aureus* bacteria lacking the SPase I SpsB that are viable and able to grow in vitro when over-expressing a native gene cassette encoding for a putative ABC transporter. This transporter apparently compensates for SpsB’s essential function by mediating alternative cleavage of a subset of proteins at a site distinct from the SpsB-cleavage site, leading to SpsB-independent secretion. This alternative secretion system also drives the main mechanism of resistance to an arylomycin-derived SpsB inhibitor, by means of mutations in a putative transcriptional regulator (cro/cI) causing over-expression of the ABC transporter. These findings raise multiple interesting biological questions. Unraveling the mechanism of SpsB-independent secretion may provide an interesting twist to the paradigm of bacterial secretion.

*Staphylococcus aureus* is an important human pathogen that can cause life-threatening invasive infections, such as bacteremia, endocarditis, pneumonia, and osteomyelitis. Because of its nominal essentiality, Type I signal peptidase (SPase I) has been investigated as a potential antibacterial target. Several factors make *S. aureus* SpsB an attractive antibiotic target [1]. First, the enzymatic domain, consisting of the serine-lysine dyad, is unique to prokaryotes and is druggable; inhibitors of the enzyme have been described previously in the literature [1]. Second, the extracellular location of the enzymatic pocket makes this target relatively accessible as it obviates the requirement of the inhibitor to traverse the bacterial membrane. Recently, derivatives of the arylomycin family of SpsB inhibitors with enhanced potency against *S. aureus* have been generated [2, 3].

A surprising observation from resistance studies performed by our group and others is that mutations in the locus encoding for the putative transcriptional regulator cro/cI confer resistance to arylomycin-derived SpsB inhibitors in *S. aureus* [3, 4]. The Cro/CI protein shares sequence similarity with lambda phage Cro, and has transcriptional repressor activity in *S. aureus* [3]. Loss-of-function mutations in the cro/cI locus are associated with over-expression of the 3-gene locus immediately downstream of cro/cI which encodes a putative ABC transporter [3, 5] (see Figure 1A). Over-expression of the ABC transporter is sufficient to confer resistance to SpsB inhibitors, whereas over-expression of the transporter with an inactivated ATPase domain is not [3]. This suggests that this resistance mechanism is ATP-dependent and possibly mediated by active transport [3]. Over-expression of the ABC transporter overcomes the *S. aureus* lethality caused by either pharmacologic inhibition or genetic ablation of SpsB, and is associated with secretion of alternatively cleaved proteins [3, 5] (see Figure 2 for a model). Thus, over-expression of the putative ABC transporter is both necessary and sufficient to compensate for the essentiality of *S. aureus* SpsB in vitro. Together these findings introduce a new concept, which challenges the common belief that SpsB is absolutely essential for viability, and which invites a number of interesting questions.
First, to what extent does the putative ABC transporter compensate for the absence of functional SpsB? Proteomic studies of the secretome of a wild-type *S. aureus* strain and an *S. aureus* strain that lacks SpsB while over-expressing the ABC transporter show that only a minor subset of proteins that are normally SpsB-cleavable is alternatively secreted [3, 5]. These proteins, which are secreted independently of SpsB, are cleaved at a site N-terminal to the canonical SpsB-cleavage site [3]. Given that the ABC transporter does not encode any domain that resembles a protease, it is feasible that one of its substrates possesses the proteolytic activity responsible for this alternative cleavage. The observation that the alternative cleavage occurs N-terminal of the SpsB cleavage site suggests the involvement of a membrane-localized protease. Although the strain lacking SpsB and over-expressing the ABC transporter appears to grow well *in vitro*, it is unable to establish infection in a mouse model [3]. Pharmacological inhibition of SpsB has been shown to reduce secretion of several *S. aureus* virulence factors [6]. Notably, when SpsB expres-

![FIGURE 1: Genomic organization of the operon of the Cro/CI and ABC transporter operon in *Staphylococcus*. (A) Functional model for the operon in *S. aureus*. The *S. aureus* cro/cI gene is a homolog of the lambda phage transcriptional repressor cro, and shares an operon with three genes encoding a putative ABC transporter (abbreviated as ABC1, ABC2, and ABC3). *S. aureus* Cro/CI functions as a transcriptional regulator, suppressing transcription of all four operon genes. The putative binding site of the Cro/CI protein presumably lies just upstream of the cro/cI gene in its promoter area. Mutations in cro/cI lead to 40-100 fold over-expression of the putative ABC transporter, causing resistance to arylomycin derivatives. Genes and promoter area are not drawn to scale. For more details see reference [3]. (B) Schematic showing the genomic organization of the cro/cI - ABC transporter operon in selected pathogenic *Staphylococcus* genomes. The intact operon is well conserved in these staphylococci. Exceptions are the lack of ABC2 and ABC3 encoding genes in 2 of 5 *S. epidermidis* genomes, and an ~15 kb insertion between ABC2 and ABC3 in 7 of 124 *S. aureus* genomes. The phyletic distribution of cro/cI and the ABC transporter genes was determined using BLAST+ [8]. The maximum-likelihood (RAXML [9], GTRGAMMA) phylogeny was constructed on the basis of a concatenated core genome alignment generated using Mugsy [10]. Nodes with ≥ 95% bootstrap support from 1,000 replicates are indicated by black diamonds. Present genes are dark gray, absent genes are white with a dotted outline, and the insertion is black. In parentheses are the numbers of *S. aureus* strains of the total examined that share the genomic organization of the representative strains shown.
sion is genetically disrupted, over-expression of the ABC transporter is not able to sufficiently restore secretion of several specific virulence factors known to contribute to establishment of infection, including adhesion molecules and secreted proteases [3]. Thus, the rather limited subset of proteins that are alternatively secreted through the ABC transporter in the absence of SpsB uncouples in vitro bacterial growth from in vivo infectivity. An interesting consideration that follows is that since S. aureus is a commensal of mammalian skin and nares [7], the strong conservation of SpsB may thus be a consequence of its essentiality for viability within these niches.

Second, by which mechanism does the alternative ABC transporter-mediated secretion pathway compensate for the essentiality of SpsB in vitro? To speculate on the answer, it would be relevant to understand the mechanism by which SpsB inhibition leads to bacterial death. Two main hypotheses, that are not mutually exclusive, can be proposed. First, in the absence of SpsB activity, cell death may be caused by accumulation of unprocessed proteins leading to disruption of membrane integrity. This hypothesis raises the possibility that the ABC transporter is directly or indirectly involved in removal of unprocessed proteins. It is possible that the transporter enables a hypothetical membrane-associated proteolytic enzyme to degrade these unprocessed membrane proteins. Second, under SpsB inhibitory conditions, cell death may occur because certain secreted proteins that are essential for viability cannot be secreted. In the context of this hypothesis, it can be speculated that in the absence of SpsB, the ABC transporter enables secretion of an individual protein or a combination of proteins that is otherwise essential. Although such proteins have not been identified as yet, this possibility also cannot be excluded. Future experiments to elucidate the mechanism by which the ABC transporter compensates for unprocessed proteins, and eventually resulting in cell death. (C) Over-expression of the putative ABC transporter (as in S. aureus with mutations in the transcriptional repressor cro/cI) compensates for a lack of SpsB activity. This transporter mediates SpsB-independent cleavage of a subset of proteins at an alternative cleavage site, leading to their secretion. This alternative secretion pathway is able to restore viability under conditions of either pharmacological SpsB inhibition (in which case it constitutes the main resistance mechanism) or genetic disruption of SpsB expression. For more details see reference [3].
reflects the lack of a functional ABC transporter, as each is essential for resistance of a *S. aureus* USA300 *cro/cI* mutant to SpsB inhibitors [3]. Second, in 7 of 124 *S. aureus* genomes analyzed, the operon has been interrupted by the obvious insertion of an ~15 kb transposable element between the *ABC2* and *ABC3* encoding genes (Figure 1B). While the gene encoding *ABC3* in these strains may no longer be under the control of the *cro/cI* promoter, it is unclear whether it can still be expressed and whether an active ABC transporter can be formed. Thus, the operon is not universally intact in all *Staphylococcus* genomes; further experimental studies are needed to determine whether insertion of the transposable element in the *cro/cI* – ABC transporter operon inactivates SpsB-independent secretion.

In conclusion, the discovery of a potential new secretion system in *S. aureus* that is able to bypass the nominal essentiality of SpsB raises a number of interesting questions. Determining the molecular mechanism of this alternative secretion pathway has the potential to provide new insights into the basic biology of bacterial secretion and to aid design of new antibacterial therapies.

**ACKNOWLEDGMENTS**

The authors would like to especially thank J. Hiroshi Morisaki, Tommy Cheung and David Arnott (Genentech, Inc.) for their contributions to defining the “alternative secretome” of *S. aureus*.

**CONFLICT OF INTEREST**

The authors have no conflict of interest.

**COPYRIGHT**

© 2017 Hazenbos et al. This is an open-access article released under the terms of the Creative Commons Attribution (CC BY) license, which allows the unrestricted use, distribution, and reproduction in any medium, provided the original author and source are acknowledged.

Please cite this article as: Wouter L.W. Hazenbos, Elizabeth Skippington and Man-Wah Tan (2017). *Staphylococcus aureus* type I signal peptidase: essential or not essential, that’s the question. Microbial Cell 4(4): 108-111. doi: 10.15698/mic2017.04.566

**REFERENCES**

1. Rao S, De Waelheyns E, Economou A, Anné J (2014). Antibiotic targeting of the bacterial secretory pathway. Biochimica et Biophysica Acta 1843: 1762-1783.

2. Therien AG, Huber JL, Wilson KE, Beaulieu P, Caron A, Claveau D, Deschamps K, Donald RG, Galgozi AM, Gallant M, Gu X, Kevin NJ, Laffleur J, Leavitt PS, Lebeau-Jacob C, Lee SS, Lin MM, Michels AA, Ogawa AM, Painter RE, Parish CA, Park YW, Benton-Perdomo L, Petchu M, Phillips JW, Powles MA, Skorey K, Tam J, Tan CM, Young K, Wong S, Waddell ST, Miesel L (2012). Broadening the spectrum of β-lactam antibiotics through inhibition of signal peptidase Type I. Antimicrobial Agents and Chemotherapy 56(9):4662-70.

3. Morisaki JH, Smith PA, Date SV, Kajihara KK, Truong CL, Modrusan Z, Yan D, Kang J, Xu M, Shah IM, Mintzer R, Kofoed EM, Cheung TK, Arnott D, Koehler MFT, Heise CE, Brown EJ, Tan MW, Hazenbos WLW (2016). A putative bacterial ABC transporter circumvents the essentiality of signal peptidase. mBio 7(5): e00412-16.

4. Craney A, Romesberg FE (2015). A putative Cro-like repressor contributes to arylomycin resistance in *Staphylococcus aureus*. Antimicrobial Agents and Chemotherapy 59(6):3066-3074. doi: 10.1128/AAC.04597-14

5. Craney A, Dix MM, Adhikary R, Cravatt BF, Romesberg FE (2015). An alternative terminal step of the general secretory pathway in *Staphylococcus aureus*. mBio 6(4): e00412-16.

6. Schallenberger MA, Niessen S, Shao C, Fowler BJ, Romesberg FE (2012). Type I signal peptidase and protein secretion in *Staphylococcus aureus*. Journal of Bacteriology 194(10):2677-2686.

7. Wertheim HF, Melles DC, Van Leeuwen W, Van Belkum A, Verbrugh HA, Nouwen JL (2005). The role of nasal carriage in *Staphylococcus aureus* infection. Lancet 365(9470):751-62.

8. Camacho C, Coulouris G, Avagyan V, Ma N, Papadopoulos J, Bealer K, et al. (2009). BLAST+: architecture and applications. BMC Bioinformatics 10: 421.

9. Stamatakis A (2006). RAxML-VI-HPC: maximum likelihood-based phylogenetic analyses with thousands of taxa and mixed models. Bioinformatics 22(21): 2688–2690.

10. Angiuoli SV, Salzberg SL (2011). Mugsy: fast multiple alignment of closely related whole genomes. Bioinformatics 27(3): 334-342.