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The inhibition of type I bacterial signal peptidase: biological consequences and therapeutic potential

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Abstract

The general secretory pathway has long been regarded as a potential antibiotic drug target. In particular, bacterial type I signal peptidase (SPase) is emerging as a strong candidate for therapeutic use. In this review, we focus on the information gained from the use of SPase inhibitors as probes of prokaryote biology. A thorough understanding of the consequences of SPase inhibition and the mechanisms of resistance that arise are essential to the success of SPase as an antibiotic target. In addition to the role of SPase in processing secreted proteins, the use of SPase inhibitors has elucidated a previously unknown function for SPase in regulating cleavage events of membrane proteins.

Graphical Abstract

Keywords

SPase; Arylomycin; Protein secretion; Antibiotic; Virulence

Clinically used antibiotics target only a handful of key cellular processes, including protein translation, cell wall synthesis, DNA replication or transcription, and more recently, membrane biogenesis.¹ While these drugs have proven remarkably effective, the rate at which bacteria develop resistance now threatens to compromise their utility. Efforts to ensure the availability of efficacious therapeutics have focused on re-optimizing current

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antibiotics to overcome resistance, identifying new antibiotics with activity against resistant bacteria, and identifying new antibiotics that target alternative processes.

One promising alternative process is the general secretory (Sec) pathway,² which functions to translocate proteins across the cytoplasmic membrane (Figure 1). Perhaps most promising of the proteins participating in Sec-mediated protein export is type I signal peptidase (SPase),³ the protease responsible for release of secreted proteins from the cytoplasmic membrane after translocation (in addition, SPase mediates the release of proteins translocated by the twin arginine translocation (TAT) pathway). Many aspects of SPase make it an attractive target, including the fact that it is essential and conserved across Gram-positive and Gram-negative bacteria, and that its active site is exposed on the outerleaflet of the cytoplasmic membrane, making it relatively accessible. In addition, while SPase is an endopeptidase, a class of target with great precedent for inhibitor development, it is a Ser-Lys dyad protease with *si* face nucleophilic attack,⁴ which functionally differentiates it from the more common Ser-His-Asp triad proteases with *re* face nucleophilic attack.⁵ Indeed, canonical serine protease inhibitors do not inhibit SPase, suggesting that specific inhibition should be possible.

There are currently five distinct groups of SPase inhibitors; two are natural products, including the arylomycin family^{6–8} and krisynomycin,⁹ and three are synthetic, including 5S penems,^{10–12} peptide substrate mimics,^{13–16} and a β -aminoketone¹⁷ (Figure 2). The current state of developing these inhibitors and targeting secretion in general has recently been reviewed.^{2,3,18} These efforts, in particular those with the arylomycins, have not only led to promising leads for antibiotic development, but also elucidated fundamental aspects of the biology associated with protein secretion and the consequences of SPase inhibition. Summarizing the current state of this knowledge is the focus of this review. While the review focuses on data generated with the arylomycins, many of the results and conclusions are likely applicable to SPase inhibition in general.

Mechanism of Action

The secretion of many, perhaps most, secreted proteins is itself not essential, and the toxic effects of SPase inhibition likely result from the accumulation of unprocessed proteins, which compromises the integrity of the cytoplasmic membrane and ultimately leads to cell lysis. For example, the activity of the arylomycins appears to depend on the level of secretion.^{19,20} During exponential growth the arylomycins are bactericidal to *E. coli* but only bacteriostatic to *Staphylococcus aureus*; however, for stationary phase cells, the reverse is true and the arylomycins are bacteriostatic to *E. coli* and bactericidal to *S. aureus*. These differences likely result from the varying demands of secretion during different phases of growth. During exponential growth, *E. coli* relies on significant levels of secretion to maintain its outer membrane and a protein rich periplasm.²¹ In contrast, *S. aureus* has neither a periplasm nor an outer membrane; however, it is known to increase secretion at stationary phase.^{22–24} Thus, SPase inhibition appears to be bactericidal when the secretion burden is high and bacteriostatic when it is lower.

The correlation between the effects of SPase inhibition and the level of protein secretion was clearly apparent in our recent examination of the activity of arylomycin against *Yersinia pestis*, which among the *Enterobacteriaceae* is atypically sensitive to the arylomycins.^{20,25} The increased sensitivity is only observed at physiological temperature (37 °C), not at lower temperatures, and it does not result from a decreased ability of the atypical (truncated or “rough”) lipopolysaccharides of the outer membrane to prevent arylomycin penetration. However, protein secretion in *Y. pestis* is known to be temperature dependent, and is increased at the physiological temperatures of its mammalian host, relative to the lower temperature of its insect vector,^{26–30} which is thought to facilitate resistance to complement and adherence to epithelial cells.^{31,32} Deletion of the gene encoding the protein whose secretion is most increased (*ail*) ablated arylomycin sensitivity, and transgenic overexpression of *malE*, which encodes the SPase processed periplasmic maltose-binding protein from *E. coli*, fully restored it. Thus, it is an increased burden of secretion at physiological temperatures that renders *Y. pestis* hypersensitive to SPase inhibition.

Interestingly, like *Y. pestis* the viability and/or virulence of many bacteria depends on increased secretion in response to particular environmental cues,^{33–36} which are typically not present during routine analysis of antibiotic susceptibility. Thus, it is possible that in the context of an actual infection, SPase inhibitors may be more potent than predicted based on traditional minimum inhibitory concentration (MIC) determination.

Response to SPase inhibition

In Gram-positive bacteria, the cell wall stress stimulon (CWSS) is involved in responding to cell wall damage and it is regulated by the two component regulator VarRS.^{37,38} The CWSS is induced by treatment with many cell wall active antibiotics, such as daptomycin, vancomycin and oxacillin.³⁹ During a study of the *S. aureus* proteome, we found that arylomycin treatment resulted in the increased production of HtrA, PrsA, and SAOUHSC_01761, which are known components of the CWSS.⁴⁰ Moreover, transcriptional profiling demonstrated that the transcription of the corresponding genes, as well as several others whose protein products are part of the CWSS, including *vraRS*, were induced upon treatment with an arylomycin.⁴¹ Strong upregulation was also observed for *vraX*, a small protein of unknown function known to be responsive to cell wall damage. Because *vraX* and the CWSS are induced by cell wall damage, and not by damage to the cytoplasmic membrane,⁴² their induction suggests that SPase inhibition precludes the correct localization (or activation, see below) of proteins that help maintain the cell wall. Indeed, many cell wall transglycosylases, transpeptidases, and hydrolases possess the canonical N-terminal leader sequences found in SPase substrates.

The evolution of resistance to SPase inhibition

In general, antibiotic resistance can evolve via mechanisms that modify or export the antibiotic or those that modify or bypass the target. The first resistance mechanism identified with SPase inhibition was target modification.²⁵ When the arylomycins were first discovered they were thought to have activity against only a limited range of bacteria (and, in fact they were thus discarded as potential drug candidates).^{6,7} However, the total

synthesis of arylomycin A-C₁₆ (Figure 2) allowed a broader survey of activity, which led to the discovery of potent activity against *Staphylococcus epidermidis*.⁴³ When arylomycin A-C₁₆-resistant *S. epidermidis* were isolated, a Ser to Pro mutation was found at position 29 or 31 of SPase.²⁵ Interestingly, sequence comparisons revealed that most of the bacteria that were previously shown to be naturally resistant to the arylomycins possessed a Pro at the position analogous to Pro29 in *S. epidermidis* SPase. Moreover, a wide variety of bacteria, including both Gram-positive and Gram-negative species, were found to encode SPases that lacked an analogous Pro residue, and the majority of these species were found to be sensitive to the arylomycins. When the corresponding Pro was mutated to Ser in the SPase of *S. aureus*, *E. coli*, or *Pseudomonas aeruginosa*, the resulting strains were sensitive to the arylomycins. Along with this data, the effect of the Ser to Pro mutation on binding affinity suggests that only a small increase in affinity is required to impart the arylomycins with activity against a broad range of important human pathogens.

Two crystal structures of an arylomycin bound to a soluble fragment of *E. coli* SPase have been reported, and they clearly elucidate the mechanism by which the Ser to Pro mutation confers resistance (Figure 3)^{44,45} The arylomycin is seen to bind in an extended β -sheet conformation that likely mimics the binding of membrane bound pre-protein substrates. The C-terminal macrocycle binds in a deep cleft and makes multiple H-bonds and hydrophobic contacts with the protein, while the C-terminal carboxyl group forms a salt bridge with the catalytic Ser and Lys residues. The peptide tail continues down a shallower cleft and forms two H-bonds with backbone residues of the protein. The critical resistance-conferring residue (Pro84 in *E. coli*) interacts with the N-terminal end of the peptidic tail and precludes the formation of an H-bond and possibly alters the trajectory of the lipid moiety as it enters the membrane.

Understanding this natural mechanism of resistance has already been useful in the design of arylomycin derivatives with an increased spectrum of activity. Specifically, in an effort to compensate for the disruptive effect of the Pro side chain, in 2011 we reported the synthesis and characterization of a set of derivatives, including several with methylene groups inserted into the peptidic tail at regions expected to interact with the resistance-conferring Pro side chain, with the expectation that this would both eliminate the unsatisfied H-bond and increase flexibility.⁴⁶ Interestingly, arylomycin derivatives **1** and **2** gained significant activity against *S. aureus*.

Although target modification of SPase is clearly a major resistance mechanism to the arylomycins, it does not fully explain the absolute sensitivity of all bacteria.²⁵ For example, *Y. pestis* SPase harbors an analogous Pro residue, but as discussed above *Y. pestis* is nonetheless sensitive to the arylomycins due to a high secretion burden. Another example is provided by *S. aureus*. Through an examination of a panel of *S. aureus* strains, we observed that despite all strains containing the resistance-conferring Pro, strains were either moderately or highly arylomycin resistant and this binary grouping was also observed in other staphylococcal species.^{41,47} Transcriptional profiling revealed that arylomycin M131 (**3**) induces the derepression of a four gene operon, SA0337–SA0340. Interestingly, sequence analysis of the clinical isolates revealed that arylomycin resistance was correlated with the sequence of SA0337, and selection for resistance *ex vivo* resulted in SA0337 mutations that result in derepression. Further characterization revealed that SA0337 is a Cro-like repressor that controls operon expression, and that the downstream genes encode a membrane protein and an ABC transporter. Based on these results, the gene locus tags SA0337–SA0340 were assigned the names *ayrRABC* for arylomycin resistance.⁴¹

An interesting but incompletely understood aspect of *ayrRABC*-mediated resistance is the role played by teichoic acids. Teichoic acids are anionic polyphosphate polymers unique to Gram-positive bacteria that can be attached to the cytoplasmic membrane (lipoteichoic acid or LTA) or to the cell wall (wall teichoic acid or WTA).⁴⁸ WTA synthesis is required for resistance in the examined clinical isolates, but not in the *ex vivo* evolved *S. aureus* strains. One possible origin of this difference may be varying levels of derepression of the *ayrRABC* operon. In the clinical isolates, resistance is correlated with different alleles of *aryR* that are differentiated by sense mutations, while in the *ex vivo* evolved strains, it is mediated by loss

of function mutations in the repressor. Thus, the operon may be only partially derepressed in the clinical isolates, and efficient secretion may thus require additional factors in the presence of high-level SPase inhibition. Indeed, in *B. subtilis*, divalent metal cations bound by the negative charges of teichoic acids are required for the proper folding of extracytoplasmic proteins.⁴⁹ Another possibility is that partial derepression, compared to full derepression, still results in cell wall stress (see above) that is synergistic with the cell wall stress caused by the absence of WTA. It is also interesting to note that LtaS, which is required for LTA synthesis, is processed by SPase at a non-canonical, internal site^{40,50,51} (see below) and that over- or under-expression of LtaS results in resistance to arylomycin M131.⁵² A more complete understanding of *ayrRABC*-mediated resistance and the role played by teichoic acids awaits further investigation.

The arylomycins as latent antibiotics

Mechanisms of arylomycin resistance clearly exist in many bacteria. Interestingly, an analysis of the large number of recently diverged SPase sequences of the coagulase negative staphylococci suggests that the Ser to Pro mutation evolved multiple times independently, suggesting a possible role for natural selection.^{25,47} As with the resistance mechanisms to β -lactams and vancomycin, which were clearly products of selection that occurred prior to therapeutic development,⁵³ this suggests that the arylomycins are what we have termed latent antibiotics, antibiotics whose spectrum is currently limited by the previous evolution of resistance, but which have had broad spectrum activity in the past, and which might be optimized to have it again. In this scenario, the Pro to Ser mutation would represent a half cycle of the arms race between arylomycin producer and susceptible strains, and it predicts that arylomycins may exist that have naturally evolved to overcome it. Indeed, in 2012, Merck disclosed a new arylomycin derivative, arylomycin D (Figure 1; also referred to as actinocarbasin), which has significantly higher activity against *S. aureus* than other arylomycins.⁹ Interestingly, one of the most notable features of arylomycin D is the presence of a β -alanine in the peptidic tail. This natural modification appears to help overcome the negative interactions mediated by the resistance-conferring Pro, and appears to be similar to the modification that imparted synthetic derivative **2** with increased activity. This apparent convergence of natural evolution and medicinal chemistry strengthens the ideas that the arylomycins are latent antibiotics and that medicinal chemistry is well suited to optimize latent antibiotics to restore broad-spectrum activity.

Interactions with other antibiotics

Methicillin resistant *Staphylococcus aureus* (MRSA) has emerged as a particularly problematic pathogen. The acquisition of the alternate penicillin binding protein PBP2a, encoded by *mecA*, protects *S. aureus* against one of the most important class of clinical antibiotics, the β -lactams. While medicinal chemistry has been able to keep up with β -lactam resistance in other bacteria by re-optimizing the β -lactam scaffold and introducing co-treatment with β -lactamase inhibitors, overcoming the resistance mediated by PBP2a has proven to be a more formidable challenge. Interestingly, co-treatment with an arylomycin restores β -lactam sensitivity. As PBP2a possesses a canonical N-terminal leader sequence, it

is interesting to speculate that SPase processing might be required for activity, although this has not been tested.

As mentioned earlier, a reduction in the secretion burden would be expected to reduce the toxicity associated with SPase inhibition. Indeed, the co-administration of sub-MIC levels of tetracycline, which inhibits translation, and thus reduces the level of proteins requiring secretion, has been shown to antagonize arylomycin activity in several bacteria.^{19,20} However, gentamicin does not reduce the level of protein translation, but rather induces translation errors. Co-administration of gentamicin results in increased sensitivity to the arylomycins, likely resulting from a synergistic accumulation of aberrant and unprocessed proteins in the cytoplasmic membrane. Thus, co-administration of an arylomycin with a β -lactam or gentamicin might represent a particularly effective therapy.

Probes of SPase function

Protein secretion plays an important role in many bacterial infections, and thus a great deal of research has focused on characterizing the secretome by identifying proteins found in the media.⁵⁴ However, at least small levels of lysis are unavoidable during growth and analysis, and this results in the release of many nonsecreted proteins into the media. While the conserved features of the N-terminal signal peptides and SPase recognition sequences has led to the bioinformatic analysis of the secretome,⁵⁵ the identified proteins are not necessarily produced under any given set of conditions, and proteins may contain difficult to identify non-canonical signal peptides. However, the availability of SPase inhibitors has made it possible to identify secreted proteins by identifying those found in the media at decreasing levels with increasing inhibitor concentration.

Using this approach, we have characterized the SPase-dependent secretome of naturally sensitive *S. epidermidis* and genetically sensitized *S. aureus*.^{40,50} In both cases, strains were treated at stationary phase with supra-MIC concentrations of arylomycin A-C₁₆ and secreted proteins were analyzed. Under these conditions, we found that the *S. epidermidis* secretome includes the proteases SspA and SspB; the nucleic acid binding protein IsaB; and the lipases GehC and GehD. The secretome also includes four peptidoglycan hydrolases, AtlE, the SsaA-like protein encoded by SERP0318, SERP2263, and IsaA; making this the major type of protein secreted by *S. epidermidis* under the conditions examined. Many more secreted proteins were detected in *S. aureus*. The most common were proteases, of which 10 were identified (including SspA, staphopain A and B, and aureolysin), but we also detected hemolysins (α , γ , and δ), four members of the leukocidin/hemolysin family of toxins, three superantigens, two lipases, and three peptidoglycan hydrolases, as well as a wide variety of other known virulence factors. There has been recent interest in inhibiting bacterial virulence,⁵⁶ and while it is unlikely that such inhibition would be refractory to the evolution of resistance (evolution works by competition for limited resources and does not require direct killing), it is interesting to note that by inhibiting the secretion of these proteins, an SPase inhibitor should reduce virulence while simultaneously killing the bacteria.

Interestingly, during these studies we also found that increased levels of the arylomycin resulted in the detection of decreased levels of the C-terminal fragments of three polytopic

membrane proteins: LtaS in both *S. epidermidis* and *S. aureus*; OatA in *S. aureus*; and BlaR1 in *S. epidermidis*. These proteins are polytopic membrane proteins with C-terminal domains and clearly do not possess canonical SPase cleavage sites. However the size of the fragments detected and sequence analysis suggested that the transmembrane domain immediately upstream of the extracytoplasmic C-terminal domain mimics a canonical signal sequence. Indeed, canonical AXA SPase recognition sequences were detected at these positions in LtaS (AFA), OatA (FDA), and BlaR1 (LMG). In addition, all three polytopic membrane proteins have catalytic domains that function on the cell surface. LTA is an essential component of Gram-positive cell walls and consists of 1,3-linked glycerolphosphate units that are anchored to the outerleaflet of the cytoplasmic membrane via the glycolipid anchor diglucosyl-diacylglycerol (Glc₂-DAG).^{57,58} The cleavage of LtaS by SPase in *S. aureus* was independently confirmed.⁵¹ During synthesis, a single glycerolphosphate (GroP) monomer, obtained from hydrolysis of the glycerolphosphate head group of membrane bound lipid phosphatidylglycerol, is first added to Glc₂-DAG by an LTA primase, to produce GroP-Glc₂-DAG, which then primes chain elongation by an LTA synthase.⁵⁹ Many bacteria, including *Listeria monocytogenes* and *B. subtilis*, encode both a primase and a soluble synthase,^{51,60} while other bacteria, including *S. epidermidis* and *S. aureus*, encode a single multidomain membrane protein, LtaS, with both primase and synthase activities. It seems possible that SPase cleavage positively or negatively regulates LtaS, with the membrane-bound LtaS acting as a primase or phosphatidylglycerol hydrolase, and the released domain required for efficient full-length polyglycerolphosphate chain synthesis. This would make the staphylococcal LtaS analogous to the two-protein system found in other bacteria. Interestingly, the non-canonical SPase cleavage site is conserved in the LtaS of other firmicutes, suggesting that it is functionally important.

Peptidoglycan remodeling is essential for cell division and is mediated by lytic transglycosylases, which catalyze the nucleophilic attack of the free C6-OH of a MurNAc moiety on the associated anomeric carbon and cleavage of the β -1,4-glycosidic linkage with the adjacent GlcNAc moiety. Many bacteria O-acylate the C6-OH group^{61,62} as a means to control remodeling, and two mechanisms have been characterized.^{63,64} The first is a two-component system, involving the integral membrane protein PatA, which transfers the acetate of acetyl-CoA across the cytoplasmic membrane, and the soluble periplasmic protein, PatB, which transfers the acetate to the target MurNAc residue.^{64,65} The second has only been observed in Gram-positive bacteria and is mediated by OatA, with an N-terminal PatA-like domain and a C-terminal O-acetyltransferase domain.⁶⁴ However, it is unclear how O-acylation occurs throughout the entirety of the peptidoglycan, including at sites that are distal from the surface site where OatA is anchored, and it is interesting to speculate that this is made possible by the SPase-mediated release of the C-terminal domain, making the *S. aureus* system more analogous to the PatA/PatB two protein system. As with LtaS, the non-canonical SPase cleavage site of OatA is conserved in a broad range of firmicutes, suggesting that it is functionally important.

The class A β -lactamase BlaZ is expressed divergently from an operon encoding BlaR1 and the repressor BlaI.^{66–68} BlaR1 assembles in the membrane and its C-terminal domain acts as a β -lactam sensor via formation of a long-lived acylated complex.⁶⁹ Once acylated, the

cytoplasmic prometalloprotease domain is activated by cleavage and in turn cleaves BlaI, resulting in the release of BlaZ from repression.⁷⁰ Cleavage within the cytoplasmic prometalloprotease domain provides an irreversible commitment to activation, and it has been suggested that the inability to turn this signal off ultimately results in cell death.⁶⁶ Thus, the observation that BlaR1 is proteolytically cleaved between the membrane and β -lactam sensor domains at an apparent internal SPase cleavage site suggests that this activity could be required to shut off induction. Alternatively, by analogy to regulated intramembrane proteolysis (RIP),^{71–73} β -lactam binding may induce cleavage between the domains and thereby activate the cytoplasmic domain. The non-canonical SPase cleavage site is conserved in the BlaR1 sequences of different strains of *S. aureus* and *S. epidermidis*, which suggests that it is functionally important.

Recent work with *B. subtilis* has also demonstrated that SPase is required for the RIP-mediated activation of the extra cytoplasmic function type σ factor σ^V in response to lysozyme challenge.⁷⁴ In this mechanism, lysozyme binds to the anti- σ factor RsiV, likely inducing a conformational change, and RsiV is then cleaved by SPase at a canonical N-terminal site (which is referred to as cleavage site-1), this event then triggers a second cleavage (at site-2) mediated by the protease RasP. These two cleavage events result in the release of σ^V and the activation of genes required for lysozyme resistance. Due to its location in the outer leaflet of the cytoplasmic membrane, SPase would appear ideally suited to participate in RIP, and it is interesting to speculate that this may couple noncanonical cleavage to regulation.

Collectively, this data suggest that the activity of SPase extends beyond processing proteins during translocation across the cytoplasmic membrane. Provocatively, bioinformatics analysis of *E. coli* suggests that many polytopic membrane proteins are encoded with internal SPase recognition sites, suggesting that non-canonical SPase substrates might be common.

Conclusions

SPase appears to be a promising target for antibiotic development. Indeed, the widespread existence of resistance mechanisms and arylomycin modifications that overcome this resistance suggest that SPase inhibition has been a focus of the arms race between bacteria, that the arylomycins are latent antibiotics that have had potent and broad spectrum activity in the past and that might be modified to regain their activity, as exemplified by derivatives **1–3**. While optimization of the inhibitors continues, in particular with the arylomycin scaffold, their availability has already enabled the elucidation of fundamental and often surprising aspects of how bacteria respond to the inhibition of secretion. The inhibitors have also helped demonstrate that SPase acts on proteins that are not secreted, but whose processing may be essential for different aspects of bacterial physiology, including cell wall maintenance and the response to different stresses. Along with a perhaps underestimated susceptibility, resulting from the secretion burden associated with an infection, this suggests that the inhibition of SPase may provide for the potent and multifaceted control of bacterial viability and virulence.

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References and notes

1. Lange RP, Locher HH, Wyss PC, Then RL. *Curr Pharm Des.* 2007; 13:3140. [PubMed: 17979755]
2. Rao CVS, De Waelheyns E, Economou A, Anne J. *Biochim Biophys Acta.* 2014; 1843:1762. [PubMed: 24534745]
3. Smitha Rao CV, Anne J. *Future Microbiol.* 2011; 6:1279. [PubMed: 22082289]
4. Paetzel M, Dalbey RE, Strynadka NC. *Nature.* 1998; 396:186. [PubMed: 9823901]
5. James, MNG. *Proteolysis and Protein Turnover.* Bond, JS.; Barrett, AJ., editors. Portland Press; London: 1993. p. 1
6. Kulanthaivel P, Kreuzman AJ, Strega MA, Belvo MD, Smitka TA, Clemens M, Swartling JR, Minton KL, Zheng F, Angleton EL, Mullen D, Jungheim LN, Klimkowski VJ, Nicas TI, Thompson RC, Peng SB. *J Biol Chem.* 2004; 279:36250. [PubMed: 15173160]
7. Schimana J, Gebhardt K, Holtzel A, Schmid DG, Sussmuth R, Muller J, Pukall R, Fiedler HP. *J Antibiot (Tokyo).* 2002; 55:565. [PubMed: 12195962]
8. Tan YX, Romesberg FE. *MedChemComm.* 2012; 3:916.
9. Therien AG, Huber JL, Wilson KE, Beaulieu P, Caron A, Claveau D, Deschamps K, Donald RG, Galgoci AM, Gallant M, Gu X, Kevin NJ, Lafleur J, Leavitt PS, Lebeau-Jacob C, Lee SS, Lin MM, Michels AA, Ogawa AM, Painter RE, Parish CA, Park YW, Benton-Perdomo L, Petcu M, Phillips JW, Powles MA, Skorey KI, Tam J, Tan CM, Young K, Wong S, Waddell ST, Miesel L. *Antimicrob Agents Chemother.* 2012; 56:4662. [PubMed: 22710113]
10. Allsop A, Brooks G, Edwards PD, Kaura AC, Southgate R. *J Antibiot (Tokyo).* 1996; 49:921. [PubMed: 8931726]
11. Black MT, Bruton G. *Curr Pharm Des.* 1998; 4:133. [PubMed: 10197037]
12. Harris DA, Powers ME, Romesberg FE. *Bioorg Med Chem Lett.* 2009; 19:3787. [PubMed: 19427205]
13. Barkocy-Gallagher GA, Bassford PJ Jr. *J Biol Chem.* 1992; 267:1231. [PubMed: 1730647]
14. Nilsson I, von Heijne G. *FEBS Lett.* 1992; 299:243. [PubMed: 1544500]
15. Buzder-Lantos P, Bockstael K, Anne J, Herdewijn P. *Bioorg Med Chem Lett.* 2009; 19:2880. [PubMed: 19362478]
16. Bruton G, Huxley A, O'Hanlon P, Orlek B, Eggleston D, Humphries J, Readshaw S, West A, Ashman S, Brown M, Moore K, Pope A, O'Dwyer K, Wang L. *Eur J Med Chem.* 2003; 38:351. [PubMed: 12750021]
17. Ollinger J, O'Malley T, Ahn J, Odingo J, Parish T. *J Bacteriol.* 2012; 194:2614. [PubMed: 22427625]
18. Segers K, Anne J. *Chem Biol.* 2011; 18:685. [PubMed: 21700205]
19. Smith PA, Romesberg FE. *Antimicrob Agents Chemother.* 2012; 56:5054. [PubMed: 22802255]
20. Steed DB, Liu J, Wasbrough E, Miller L, Halasohoris S, Miller J, Somerville B, Hershfield JR, Romesberg FE. *Antimicrob Agents Chemother.* 2015
21. Song C, Kumar A, Saleh M. *Genomics Proteomics Bioinformatics.* 2009; 7
22. Ziebandt AK, Weber H, Rudolph J, Schmid R, Hoper D, Engelmann S, Hecker M. *Proteomics.* 2001; 1:480. [PubMed: 11681202]
23. Vandenesch F, Kornblum J, Novick RP. *J Bacteriol.* 1991; 173:6313. [PubMed: 1717437]
24. Ravipaty S, Reilly JP. *Mol Cell Proteomics.* 2010; 9:1898. [PubMed: 20418541]
25. Smith PA, Roberts TC, Romesberg FE. *Chem Biol.* 2010; 17:1223. [PubMed: 21095572]
26. Brubaker, RR. *Mol Med Microbiol.* Sussman, M., editor. Academic Press; London, UK: 2002. p. 2033

27. Chromy BA, Choi MW, Murphy GA, Gonzales AD, Corzett CH, Chang BC, Fitch JP, McCutchen-Maloney SL. *J Bacteriol.* 2005; 187:8172. [PubMed: 16291690]
28. Pieper R, Huang ST, Clark DJ, Robinson JM, Parmar PP, Alami H, Bunai CL, Perry RD, Fleischmann RD, Peterson SN. *Proteomics.* 2008; 8:1442. [PubMed: 18383009]
29. Pieper R, Huang ST, Parmar PP, Clark DJ, Alami H, Fleischmann RD, Perry RD, Peterson SN. *BMC Microbiol.* 2010; 10:30. [PubMed: 20113483]
30. Pieper R, Huang ST, Robinson JM, Clark DJ, Alami H, Parmar PP, Perry RD, Fleischmann RD, Peterson SN. *Microbiology.* 2009; 155:498. [PubMed: 19202098]
31. Bartra SS, Styer KL, O'Bryant DM, Nilles ML, Hinnebusch BJ, Aballay A, Plano GV. *Infect Immun.* 2008; 76:612. [PubMed: 18025094]
32. Kolodziejek AM, Sinclair DJ, Seo KS, Schnider DR, Deobald CF, Rohde HN, Viall AK, Minnich SS, Hovde CJ, Minnich SA, Bohach GA. *Microbiology.* 2007; 153:2941. [PubMed: 17768237]
33. Lee VT, Schneewind O. *Genes Dev.* 2001; 15:1725. [PubMed: 11459823]
34. Rieder G, Fischer W, Haas R. *Curr Opin Microbiol.* 2005; 8:67. [PubMed: 15694859]
35. Sandkvist M. *Infect Immun.* 2001; 69:3523. [PubMed: 11349009]
36. Stathopoulos C, Hendrixson DR, Thanassi DG, Hultgren SJ, St Geme JW 3rd, Curtiss R 3rd. *Microbes Infect.* 2000; 2:1061. [PubMed: 10967286]
37. Kuroda M, Kuroda H, Oshima T, Takeuchi F, Mori H, Hiramatsu K. *Mol Microbiol.* 2003; 49:807. [PubMed: 12864861]
38. Utaida S, Dunman PM, Macapagal D, Murphy E, Projan SJ, Singh VK, Jayaswal RK, Wilkinson BJ. *Microbiology.* 2003; 149:2719. [PubMed: 14523105]
39. Wilkinson BJ, Muthaiyan A, Jayaswal RK. *Curr Med Chem Anti-Infect Agents.* 2005; 4:259.
40. Schallenger MA, Niessen S, Shao C, Fowler BJ, Romesberg FE. *J Bacteriol.* 2012; 194:2677. [PubMed: 22447899]
41. Craney A, Romesberg FE. *Antimicrob Agents Chemother.* 2015; 59:3066–3074. [PubMed: 25753642]
42. Muthaiyan A, Silverman JA, Jayaswal RK, Wilkinson BJ. *Antimicrob Agents Chemother.* 2008; 52:980. [PubMed: 18086846]
43. Roberts TC, Smith PA, Cirz RT, Romesberg FE. *J Am Chem Soc.* 2007; 129:15830. [PubMed: 18052061]
44. Paetzel M, Goodall JJ, Kania M, Dalbey RE, Page MG. *J Biol Chem.* 2004; 279:30781. [PubMed: 15136583]
45. Luo C, Roussel P, Dreier J, Page MG, Paetzel M. *Biochemistry.* 2009; 48:8976. [PubMed: 19655811]
46. Roberts TC, Schallenger MA, Liu J, Smith PA, Romesberg FE. *J Med Chem.* 2011; 54:4954. [PubMed: 21630667]
47. Smith PA, Powers ME, Roberts TC, Romesberg FE. *Antimicrob Agents Chemother.* 2011; 55:1130. [PubMed: 21189343]
48. Brown S, Santa Maria JP Jr, Walker S. *Annu Rev Microbiol.* 2013; 67:313. [PubMed: 24024634]
49. Hyyrylainen HL, Vitikainen M, Thwaite J, Wu H, Sarvas M, Harwood CR, Kontinen VP, Stephenson K. *J Biol Chem.* 2000; 275:26696. [PubMed: 10871614]
50. Powers ME, Smith PA, Roberts TC, Fowler BJ, King CC, Trauger SA, Siuzdak G, Romesberg FE. *J Bacteriol.* 2011; 193:340. [PubMed: 21075926]
51. Wormann ME, Reichmann NT, Malone CL, Horswill AR, Grundling A. *J Bacteriol.* 2011; 193:5279. [PubMed: 21784926]
52. Meredith TC, Wang H, Beaulieu P, Grundling A, Roemer T. *Mob Genet Elements.* 2012; 2:171. [PubMed: 23094235]
53. D'Costa VM, King CE, Kalan L, Morar M, Sung WW, Schwarz C, Froese D, Zazula G, Calmels F, Debruyne R, Golding GB, Poinar HN, Wright GD. *Nature.* 2011; 477:457. [PubMed: 21881561]
54. Armengaud J, Christie-Oleza JA, Clair G, Malard V, Duport C. *Expert Rev Proteomics.* 2012; 9:561. [PubMed: 23194272]
55. Petersen TN, Brunak S, von Heijne G, Nielsen H. *Nat Methods.* 2011; 8:785. [PubMed: 21959131]

56. McShan AC, De Guzman RN. Chem Biol Drug Des. 2015; 85:30. [PubMed: 25521643]
57. Fischer, W. Bacterial Cell Wall. Ghuysen, JM.; Hakenbech, R., editors. Elsevier Science; 1994. p. 119
58. Grundling A, Schneewind O. J Bacteriol. 2007; 189:2521. [PubMed: 17209021]
59. Webb AJ, Karatsa-Dodgson M, Grundling A. Mol Microbiol. 2009; 74:299. [PubMed: 19682249]
60. Sutcliffe IC. Mol Microbiol. 2011; 79:553. [PubMed: 21255102]
61. Ghuysen JM, Strominger JL. Biochemistry. 1963; 2:1119. [PubMed: 14087370]
62. Johannsen, L.; Labinschinski, H.; Burghaus, P.; Giesbreecht, P. The Target of Penicillin: International FEMS Symposium on the Murein Sacculus of Bacterial Cell Walls. Hakenbech, R.; Hölte, J.; Labinschinski, H., editors. Walter de Gruyter and Co; Berlin: 1983. p. 261
63. Clarke, AJ.; Strating, H.; Blackburn, NT. Glycomicrobiology. Doyle, R., editor. Plenum Publishing Co. Ltd; New York: 2000. p. 187
64. Moynihan PJ, Clarke AJ. Int J Biochem Cell Biol. 2011; 43:1655. [PubMed: 21889603]
65. Moynihan PJ, Clarke AJ. J Biol Chem. 2010; 285:13264. [PubMed: 20178982]
66. Gregory PD, Lewis RA, Curnock SP, Dyke KG. Mol Microbiol. 1997; 24:1025. [PubMed: 9220009]
67. Sharma VK, Hackbarth CJ, Dickinson TM, Archer GL. J Bacteriol. 1998; 180:2160. [PubMed: 9555900]
68. Hiramatsu K. Microbiol Immunol. 1995; 39:531. [PubMed: 7494490]
69. Golemi-Kotra D, Cha JY, Meroueh SO, Vakulenko SB, Mobashery S. J Biol Chem. 2003; 278:18419. [PubMed: 12591921]
70. Zhang HZ, Hackbarth CJ, Chansky KM, Chambers HF. Science. 2001; 291:1962. [PubMed: 11239156]
71. Brown MS, Ye J, Rawson RB, Goldstein JL. Cell. 2000; 100:391. [PubMed: 10693756]
72. Weihofen A, Martoglio B. Trends Cell Biol. 2003; 13:71. [PubMed: 12559757]
73. Urban S. Nat Rev Microbiol. 2009; 7:411. [PubMed: 19421188]
74. Hastie JL, Williams KB, Sepulveda C, Houtman JC, Forest KT, Ellermeier CD. PLoS Genet. 2014; 10:e1004643. [PubMed: 25275625]

Figure 1.

SPase functions to release proteins translocated across the cytoplasmic membrane by the general Sec (or TAT) pathway. Conserved features of the N-terminal leader sequence that target a protein to the general Sec pathway are shown; the canonical SPase cleavage site, AXA, is also shown.

Figure 2.

SPase inhibitors. Four families of arylomycin natural products exist, and except for arylomycin D, for which only a single member has been identified, each family member is differentiated by the specific lipid tail attached. Synthetic derivatives with C₁₆ tails are shown.

Figure 3.

Crystal structure of an A family arylomycin bound to a soluble domain of *E. coli* SPase. H-bonds are shown in green and the putative H-bond lost upon mutation of Ser⁸⁴ to Pro is shown in red.