An Alternative Terminal Step of the General Secretory Pathway in *Staphylococcus aureus*

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ABSTRACT **Type I signal peptidase (SPase) is essential for viability in wild-type bacteria because the terminal step of the bacterial general secretory pathway requires its proteolytic activity to release proteins from their membrane-bound N-terminal leader sequences after translocation across the cytoplasmic membrane. Here, we identify the *Staphylococcus aureus* operon *ayrRABC* (SA0337 to SA0340) and show that once released from repression by AyrR, the protein products AyrABC together confer resistance to the SPase inhibitor arylomycin M131 by providing an alternate and novel method of releasing translocated proteins. Thus, the derepression of *ayrRABC* allows cells to bypass the essentiality of SPase. We demonstrate that AyrABC functionally complements SPase by mediating the processing of the normally secreted proteins, albeit in some cases with reduced efficiency and either without cleavage or via cleavage at a site N-terminal to the canonical SPase cleavage site. Thus, *ayrRABC* encodes a secretion stress-inducible alternate terminal step of the general secretory pathway.**

IMPORTANCE **Addressing proteins for proper localization within or outside a cell in both eukaryotes and prokaryotes is often accomplished with intrinsic signals which mediate membrane translocation and which ultimately must be removed. The canonical enzyme responsible for the removal of translocation signals is bacterial type I signal peptidase (SPase), which functions at the terminal step of the general secretory pathway and is thus essential in wild-type bacteria. Here, we identify a four-gene operon in *S. aureus* that encodes an alternate terminal step of the general secretory pathway and thus makes SPase nonessential. The results have important implications for protein secretion in bacteria and potentially for protein trafficking in prokaryotes and eukaryotes in general.**

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The proper localization of many proteins requires their translocation across one or more membranes. The general secretory (Sec) pathway, conserved throughout bacteria, is the canonical translocation pathway and is responsible for translocating the vast majority of secreted proteins across the cytoplasmic membrane. Like other general translocation pathways, Sec requires the synthesis of its cargo as preproteins with N-terminal signal peptides, which target them to the Sec machinery and from which the mature protein must be released after translocation (1, 2). The enzyme responsible for the liberation of most mature proteins translocated by Sec is type I signal peptidase (SPase) (3–5). Accordingly, SPase has been demonstrated to be essential in both Gram-positive and Gram-negative bacteria. *Staphylococcus aureus* is a striking example of the importance of SPase, as it mediates the secretion of a diverse range of virulence factors, including proteins required for adhesion and colonization, evasion of the host immune response, scavenging of nutrients and minerals from the environment, and dissemination (6).

The arylomycin family of natural products are potent inhibitors of SPase (7–10). As part of an effort to develop the arylomycins as therapeutics, we and others have been exploring the optimization of their spectrum of activity (11–13). An arylomycin with particularly promising activity against *S. aureus* is arylomycin M131 (Fig. 1A), which was disclosed by Merck in 2012 (13). We have also developed the arylomycins as chemical biology probes to assess secretion in different bacteria, including *Staphylococcus epidermidis* (14) and *S. aureus* (6). As part of these efforts, we recently demonstrated that *S. aureus* responds to arylomycin-mediated SPase inhibition by increasing expression of the four adjacent genes, SA0337 to SA0340, and that arylomycin resistance is conferred by loss-of-function mutations in SA0337 (15).

Here, we demonstrate that the genes SA0337 to SA0340 constitute an operon and that SA0337 is a transcriptional repressor that controls its expression. Remarkably, derepression of these genes bypasses the need for SPase, rendering SPase nonessential and suggesting that the function of the operon is to mediate an alternate process by which translocated proteins are released from the cytoplasmic membrane. We have thus named the repressor gene *ayrR* and the downstream genes *ayrABC* for their role in arylomycin resistance. Sequence analysis identifies AyrA as a 6-transmembrane-domain protein of unknown function (DUF3169/PF11368) and AyrB and AyrC as two domains of an ABC transporter, and we demonstrate that all three are required to bypass SPase. Finally, we demonstrate that AyrABC is

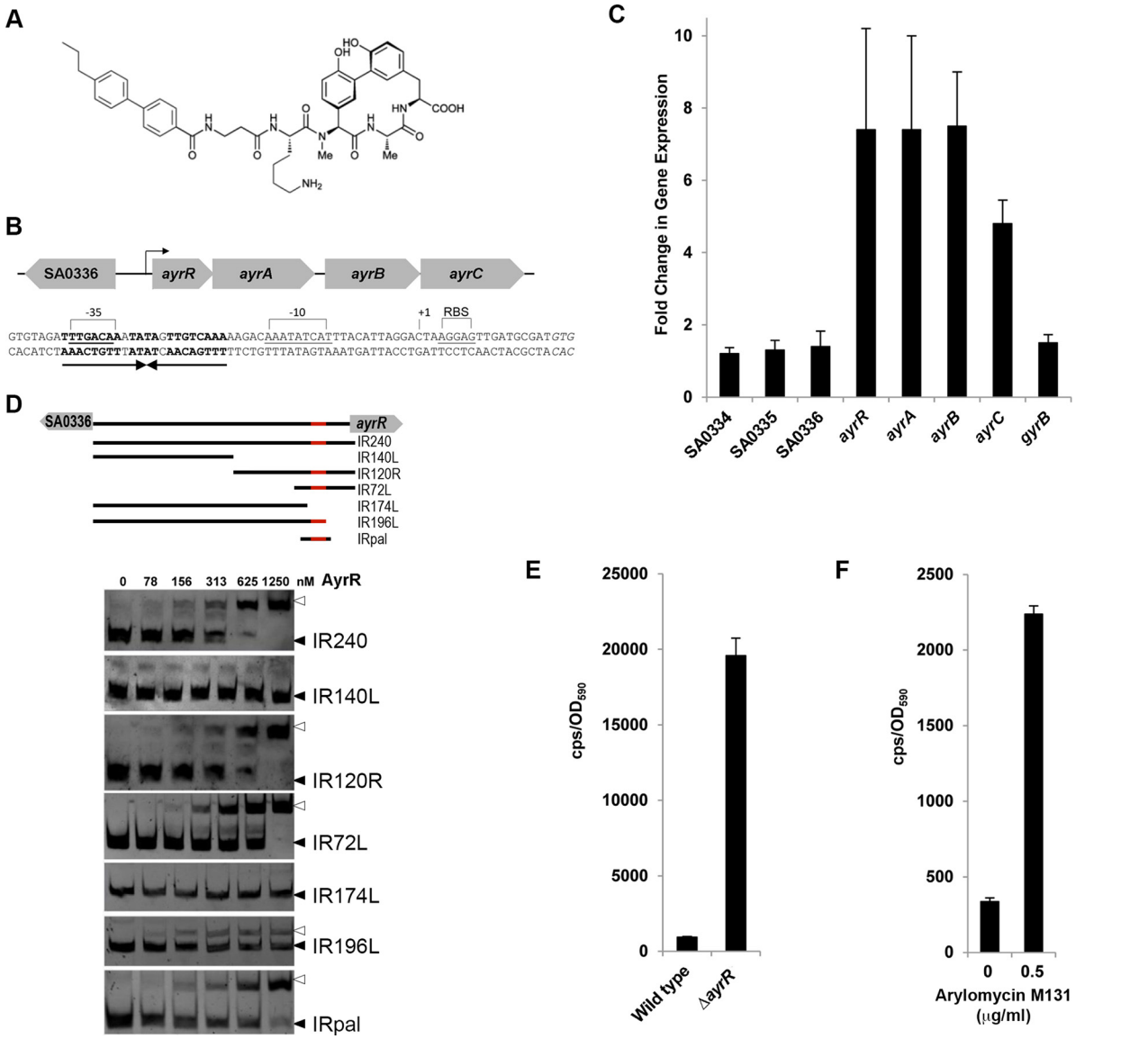

FIG 1 AyrR is a transcriptional repressor of itself and three downstream open reading frames. (A) Chemical structure of the SPase inhibitor arylomycin M131. (B) Genomic organization of the *ayrRABC* operon (*ayrR*, SA0337; *ayrA*, SA0338; *ayrB*, SA0339; *ayrC*, SA0340) and the region immediately upstream that includes SA0336. Below is the sequence of the putative promoter region upstream of *ayrR* ending with its GTG translational start site. A 22-nt palindromic repeat (arrows) is located 43 to 65 bp upstream of the start site. The positions of the putative −35 and −10 regions and ribosome binding site (RBS) are indicated in the coding strand. The transcriptional start site is marked as +1, corresponding to 18 nt upstream from the translational start site. (C) RT-PCR analysis of the *ayrRABC* operon and divergent genes (SA0334 to SA0336). Fold change in gene expression in ARC0001 with respect to N315. *gyrB* was used as an external control, and gene expression was normalized using *gmk*. See Table S1 in the supplemental material for primer sequences. (D) Gel shifts performed with 30 ng of each DNA probe and an increasing concentration of AyrR as indicated at the top. The region of DNA corresponding to each gel shift probe is shown above the gel data, with the AyrR binding site shown in red. Filled triangles denote free DNA probe, and open triangles denote the indicated DNA-AyrR complexes. All DNA probes were amplified from wild-type N315 genomic DNA with the exception of IRpal, which was created by annealing synthetic oligonucleotides. See Table S1 for primer sequences. (E) Luminescence from the AyrR promoter (expressed from pARC1) in wild-type N315 and N315ΔSA0336-ayrRABC (labeled Δ*ayrR*), expressed as counts per second per unit of optical density at 590 nm (cps/OD_{590}). (F) Luminescence from N315 harboring pARC1 in the presence and absence of arylomycin M131 (0.5× MIC).

able to mediate secretion of the proteins normally processed by SPase, although in some cases with reduced efficiency, but it does so either with the signal peptide still attached or after cleavage at a site within the signal peptide.

***ayrRABC* is an operon, and AyrR is a repressor that regulates its expression.** The stop and start codons of *ayrR* and *ayrA* overlap, as do those of *ayrB* and *ayrC*, while the stop and start codons of *ayrA* and *ayrB* are separated by only 24 bp (Fig. 1B), suggesting that they are all part of a cotranscriptionally regulated operon. Using reverse transcription-PCR (RT-PCR), we examined transcription of *ayrRABC* in the arylomycin-resistant strain ARC0001, which harbors a nonsense mutation in *ayrR* (15), and its parental wild-type strain N315. As expected, no differences in transcript levels were observed for control genes (SA0334 to SA0336 and

gyrB), but transcript levels of *ayrR*, *ayrA*, *ayrB*, and *ayrC* in ARC0001 were each increased ~8-fold compared to those in the wild type (Fig. 1C). Moreover, analysis of our previously published RNA-Seq data (15) reveals reads that when overlapped align continuously across the entire *ayrRABC* locus, including the intergenic sequences (see Fig. S1 in the supplemental material). Collectively, these results suggest that *ayrRABC* is an operon and demonstrate that the mutation in *ayrR* that renders *S. aureus* resistant to SPase inhibition results in operon derepression.

ayrR encodes a helix-turn-helix motif protein annotated as an XRE family transcriptional regulator with homology to the phage λ Cro repressor. Sequence analysis revealed an almost perfect 22-nucleotide (nt) palindrome, TTTGACAAATATAGTTGTCAAA, upstream of *ayrR* which overlaps the −35 promoter element (Fig. 1B). Such palindromes commonly compose the binding site of transcriptional regulators (16), and Cro regulates its own transcription by binding such a palindrome (17, 18). To determine if AyrR binds its upstream palindrome, a gel shift assay was employed using various DNA fragments (Fig. 1D). The data clearly demonstrate that AyrR selectively binds the palindrome.

To test the functional significance of this binding, we constructed the transcriptional reporter plasmid pARC1, which harbors the intergenic region between SA0336 and *ayrR* upstream of the genes encoding luciferase (*luxCDABE*) (19). Wild-type N315 and a strain lacking the entire region from SA0336 to *ayrC* (N315ΔSA0336-*ayrRABC*) were then transformed with either the empty *luxCDABE* vector or pARC1, and luminescence was assayed in actively growing cultures (Fig. 1E). Only in the case of N315ΔSA0336-*ayrRABC* transformed with pARC1 did we observe high-intensity luminescence. Luminescence was significantly lower for N315 harboring pARC1, consistent with repression by the genomically encoded AyrR protein. As an additional control, the ability of SPase inhibition to release repression was tested by conducting the same experiment in the presence of a subinhibitory concentration of arylomycin M131 (0.5× MIC). Two hours after arylomycin addition, the luminescence signal observed with wild-type N315 cells harboring pARC1 was 7-fold higher than that observed in the absence of arylomycin (Fig. 1F). These results confirm that AyrR acts as a repressor of *ayrRABC* and that SPase inhibition induces derepression.

Each gene of the *ayrRABC* operon contributes to tolerating SPase inhibition. *ayrA* encodes a putative membrane protein, predicted to possess a DUF3169 domain of unknown function. Proteins in the DUF3169 family are found in both *Staphylococcus* and *Streptococcus* species, with one homolog present per genome in sequenced strains, and while they share only ~30% sequence identity, six predicted transmembrane domains and a D-E(a/g)E motif located in the loop connecting the fourth and fifth predicted transmembrane segments are highly conserved. Interestingly, each *ayrA* homolog identified appears to be immediately downstream of a gene that is homologous to *ayrR*. The downstream genes *ayrB* and *ayrC* are predicted to encode the two domains of a type 2 family ABC transporter, with *ayrC* encoding the transporter domain and *ayrB* encoding the ATP-binding cassette domain (conserved domain cd03230).

The contribution of each gene to arylomycin resistance was assessed via gene deletion. Although *ayrA* could be deleted in an otherwise wild-type strain (N315), we were unable to delete *ayrA* in ARC0001. This suggests that the derepression of *ayrBC* is toxic in the absence of AyrA. Thus, to test whether *ayrA* is required to tolerate SPase inhibition, we attempted to evolve arylomycin resistance in N315Δ*ayrA*. As a control, we constructed and analyzed a strain lacking SA0336, which is predicted to encode a hypothetical protein and which is not part of the *ayrRABC* operon. High-level arylomycin M131 resistance (>16 μg/ml) was evolved in N315ΔSA0336 with frequencies indistinguishable from the wild-type parental strain (2.0×10^{-8}) (15) (see Table S2 in the supplemental material). In addition, sequencing revealed that 8 out of 8 of the resistant strains examined contained mutations in *ayrR* (see Table S3 in the supplemental material), similar to the behavior observed with wild-type cells (15). In contrast, N315Δ*ayrA* developed high-level resistance with a significantly reduced frequency (2.9×10^{-10}) (see Table S2), and for the single resistant clone isolated, sequencing revealed a wild-type *ayrR*.

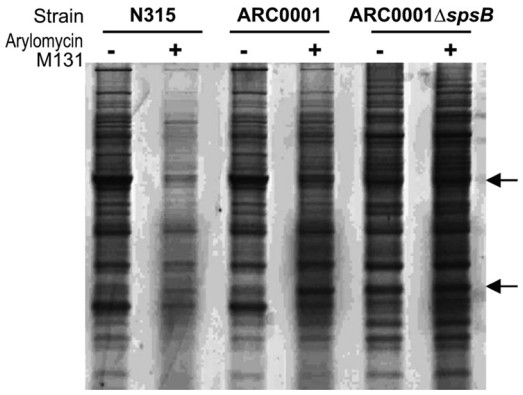

FIG 2 The extracellular proteomes of N315, ARC0001, and ARC0001Δ*spsB* in the absence and presence of arylomycin M131. To minimize cell lysis, strains were grown until wild-type N315 reached an OD_{590} of 4.0, corresponding to late exponential growth. Strains were then balanced to an OD_{590} of 4.0, pelleted by centrifugation, and washed twice with medium before resuspension in fresh medium. Strains were then grown in the presence or absence of 4× MIC of arylomycin M131 (N315) for 20 min. Cells were pelleted and the supernatant was collected. Proteins in the supernatant were precipitated overnight with 10% (wt/vol) TCA, washed with acetone, resuspended in 1× loading dye, and visualized by SDS-PAGE. Arrows denote bands where significant differences are observed in the extracellular proteomes of wild-type N315 and ARC0001Δ*spsB*.

Deletion of *ayrBC* in ARC0001 (ARC0001Δ*ayrBC*) resulted in full resensitization to arylomycin M131, demonstrating that the ABC transporter is required to tolerate SPase inhibition (see Table S2 in the supplemental material). Moreover, deletion of *ayrBC* in N315 (N315Δ*ayrBC*) results in the same reduced frequency of resistance to arylomycin M131 that was observed with N315Δ*ayrA* (see Table S2), and again, sequencing revealed wild-type *ayrR* in the single resistant mutant isolated. Collectively, these results demonstrate that each member of the *ayrRABC* operon is required to tolerate SPase inhibition.

Derepression of *ayrRABC* bypasses the requirement for SPase. Because derepression of *ayrRABC* in *S. aureus* confers high-level resistance to SPase inhibition, we speculated that it may render SPase nonessential. Using the same chromosomal integration approach, we attempted to delete the SPase gene *spsB* in ARC0001 and its parental wild-type strain, N315. Not surprisingly, attempts to delete *spsB* in the wild-type strain resulted in the recovery of the wild-type sequence. In contrast, the ARC0001Δ*spsB* strain was viable. In addition to confirming the absence of *spsB* at its chromosomal locus via sequencing, we demonstrated that *spsB* was not present elsewhere

TABLE 1 Comparison of the secreted proteomes of wild-type N315 and ARC0001Δ*spsB*

Locus	Name	Description	Fold change[a]	SPase signal sequence[b]
SA2285		Putative surface protein	0.13 ± 0.01	MRDKKGPVNKRVDFLSNKLNKYSIRKFTVGTASILI GSLMYLGTQQEAEA
SA1758	Sak	Staphylokinase	0.17 ± 0.01	MLKRSLLFLTVLLLLFSFSSITNEVSA
SA1754	Scn	Staphylococcal complement inhibitor	0.26 ± 0.10	MKIRKSILAGTLAIVLASPLVTNLDKNEAQA
SA0620		Peptidase M23	0.28 ± 0.03	MKKLAFAITATSGAAAFLTHHDAQA
SA1813		Leukocidin/hemolysin toxin family	0.30 ± 0.04	MKNKKRVLIASSLSCAILLLSAATTQANSAHK
SA1268	EbhB	Extracellular matrix-binding protein	0.32 ± 0.05	MNYRDKIQKFSIRKYTVGTFSTVIATLVFLGFNTSQAHA
SA2431	IsaB	Immunodominant staphylococcal antigen B	0.34 ± 0.04	MNKTSKVCVAATLALGTLIGVTVVENSAPTSKQAQA
SA2097		Secretory antigen precursor	0.34 ± 0.08	MKKLVTATTLTAGIGTALVGQAHHADA
SA1725	SspP	Staphopain A	0.36 ± 0.08	MKRNFPKLIALSLIFSLSVTPIANA
SA0107	Spa	Immunoglobulin G-binding protein A	0.37 ± 0.06	MKKKNIYSIRKLGVGIASVTLGTLLISGGVTPAANA
SA0587	PsaA	Metal-binding protein PsaA	0.38 ± 0.04	MKKLVPLLLALLLLVAACGTGGKQS
SA0914		Chitinase	0.38 ± 0.06	MNKLLQSLSALGVSATLVTPNLNADA
SA2074	ModA	Probable molybdate-binding protein	0.40 ± 0.08	MKMKRFIAIVMALFLVLAGCSNS
SA0222	Coa	Staphylocoagulase precursor	0.45 ± 0.16	MKKQIISLGALAVASSLFTWDNKADA
SA2208	HlgC	Gamma-hemolysin component C	0.45 ± 0.05	MLKNKILATTLSVSLLAPLANPLLENAKA
SA1761	Sep	Enterotoxin P	0.47 ± 0.16	MSKIKKTTFILLSFIALTLITSPFVNCSEK
SA1755	Chp	Chemotaxis inhibitory protein	0.47 ± 0.10	MKKKLATTVLALSFLTAGISTHHHSAKA
SA0905	Atl	Bifunctional autolysin	0.47 ± 0.08	MAKKFNYKLPSMVALTLVGSAVTAHQVQA
SA2356	IsaA	Probable transglycosylase IsaA	0.51 ± 0.05	MKKTIMASSLAVALGVTGYAAGTGHQAHA
SA2206	Sbi	Immunoglobulin-binding protein Sbi	0.52 ± 0.04	MKNKYISKLLVGAATITLATMISNGEAKA
SA0570		Hypothetical	0.55 ± 0.04	MKKLLTASIIACSVVMGVGLVNTSAEA
SA1964	FmtB	Methicillin resistance protein	0.56 ± 0.11	MNLFRQQKFSIRKFNVGIFSALIATVTFISTNPTTASA
SA0265	LytM	Glycyl-glycine endopeptidase	0.57 ± 0.03	MKKLTAAAIATMGFATFTMAHQADA
SA2353	SsaA1	Staphylococcal secretory antigen SsaA1	0.61 ± 0.12	MKKIVTATIATAGLATIAFAGHDAQA
SA2093	SsaA2	Staphylococcal secretory antigen ssaA2	0.61 ± 0.05	MKKIATATIATAGFATIAIASGNQAHA
SA1000		Fibrinogen-binding protein	0.62 ± 0.20	MKKNFIGKSILSIAAISLTVSTFAGESHA
SAP010	BlaZ	Beta-lactamase	0.63 ± 0.10	MKKLIFLVIALVLSACNSNSSHA
SA1003		Fibrinogen-binding protein	0.63 ± 1.09	MKNKLIAKSLLTIAAIGITTTTIASTADA
SA0394		Hypothetical	0.66 ± 0.05	MRENFKLRKMKVGLVSVAITMLYIMTNGQAEA
SA2437		*N*-Acetylmuramoyl-L-alanine amidase	0.74 ± 0.07	MPKNKILIYLLSTTLVLPTLVSPTAYA
SA0744	Emp	Extracellular matrix protein-binding protein	0.82 ± 0.21	MKKKLLVLTMSTLFATQLINSNHAKA
SA0393	Set15	Exotoxin 15	0.88 ± 0.05	MKLKNIAKASLALGILTTGMITTTAQPVKA
SA0520	SdrD	Serine-aspartate repeat-containing protein D	0.90 ± 0.29	MLNRENKTAITRKGMVSNRLNKFSIRKYTVGTASILVG TTLIFGLGNQEAKA
SA1751	Map	Truncated map-w protein	1.15 ± 0.21	MKFKSLITTTLALGVIASTGANFNTNEASA
SA0908		Hypothetical	1.47 ± 0.42	MNKFLKYFLILLALVLIVVPIVFATLLFKTSQDA
SA0710		Peptidase M23	1.58 ± 0.16	MKKTLTVTVSSVLAFLALNNAAHA
SA0663		Hypothetical	6.31 ± 5.41	MNTKYFLAVGAVASVLTLGACGNSNS
SA0521	SdrE	Serine-aspartate repeat-containing protein E	8.13 ± 5.15	MINRDNKKAITKKGMISNRLNKFSIRKYTVGTASILVG TTLIFGLGNQEAKA
SA1659	PrsA	Peptidylprolyl isomerase	16.53 ± 6.0	MKMINKLIVPVTASALLLGACGASA

[a] Reported as the average for all detected tryptic peptides for a given protein. Data are averages and standard errors of the means for three independent biological samples.
[b] Underlined amino acids correspond to tryptic peptides detected in ARC0001Δ*spsB* which contain part of the SPase signal sequence.

in the genome of ARC0001Δ*spsB* via PCR and RT-PCR of genomic DNA and RNA, respectively, using primers internal to the *spsB* gene (see Table S4 in the supplemental material).

Deletion of *spsB* was also verified phenotypically via an examination of the susceptibility of N315 and ARC0001Δ*spsB* to gentamicin and the β-lactam antibiotics cefoxitin, oxacillin, and penicillin G. Previously, we and others demonstrated that the activity of these antibiotics is uniquely synergistic with that of the arylomycins (13, 20). As controls, we also examined susceptibilities to CCCP, vancomycin, daptomycin, erythromycin, trimethoprim, and tetracycline. For each control antibiotic tested, the observed susceptibilities of N315 were virtually identical to those observed for N315 and ARC0001 (15). However, the susceptibility of ARC0001Δ*spsB* to gentamicin and each β-lactam was 8- to 64-fold greater than that of N315 or ARC0001 (see Table S5 in the supplemental material), consistent with the absence of SPase activity.

The activity of the *ayrRABC* complements SPase deletion. We next evaluated protein secretion in N315, ARC0001, and ARC0001Δ*spsB* using one-dimensional (1D) SDS-PAGE (Fig. 2). Identical patterns of secretion were observed for the wild type and ARC0001, which suggests that ARC0001 still employs SPase even though the *ayrRABC* operon is derepressed. In contrast, a small but clearly significant alteration in the pattern of secretion was apparent for ARC0001Δ*spsB*. To confirm that this altered pattern of secretion was the result of the loss of SPase activity, secretion was examined in the presence of arylomycin M131 (4× MIC). As expected, wild-type cells showed drastically reduced levels of secretion in the presence of the arylomycin. However, treatment of ARC0001 cells with the arylomycin did not reduce secretion and resulted in a secretion pattern indistinguishable from that of ARC0001Δ*spsB*, clearly revealing that the altered pattern does indeed result from the absence of SPase activity. Moreover, the pattern and quantity of secreted proteins ob-

served with ARC0001Δ*spsB* were unchanged by the addition of the arylomycin, confirming that secretion did not depend on SPase function.

The secreted proteomes of ARC0001Δ*spsB* and N315 were next compared in more detail by isolating proteins from the medium, digesting with trypsin, using reductive dimethylation (ReDiMe) to label the wild-type fragments with heavy isotopes and label the ARC0001Δ*spsB* fragments with light isotopes, and then analyzing the combined samples via multidimensional mass spectrometry (MudPIT) (21, 22). In total, tryptic peptides corresponding to 38 proteins that are encoded with signal peptides were identified (Table 1), which is consistent with previous studies of the *S. aureus* secretome (6). The quantity of secreted proteins was then determined from the ratio of parent ion peak areas, which demonstrated that while tryptic peptides for the same proteins were detected from each strain, several were detected at different levels (Table 1). Sixteen of the 38 secreted proteins were detected in both strains at similar levels (within 2-fold), while 20 were detected at 2- to 8-fold-reduced levels (for example, the proteases staphylokinase and SspP, the complement inhibitor Sak, and a hypothetical surface protein SA2285). Two proteins (SA0663 and SdrE) were detected at 6- to 8-fold-elevated levels in the Δ*spsB* strain, while PrsA was detected at a 16-fold-elevated level. PrsA is a peptidyl-prolyl isomerase involved in protein folding of secreted proteins in the extracellular environment (23), and we previously demonstrated that it is upregulated at the transcriptional level by SPase inhibition. Examination of signal sequences revealed no obvious correlation with observed differences in secretion.

As expected for secretion mediated by SPase, none of the tryptic peptides isolated from the wild-type sample contained any part of the signal peptide; however, peptide fragments for eight of the proteins were found with N termini that precisely matched the predicted SPase cleavage site (see Fig. S3 in the supplemental material). In contrast, with ARC0001Δ*spsB*, tryptic peptides containing portions of the N-terminal leader peptides of 12 of the 38 proteins were detected (Table 1; also, see Fig. S3 in the supplemental material). These data suggest that AyrABC mediates secretion of the same proteins as does SPase but either via cleavage at a more N-terminal site or perhaps by extricating the entire intact preprotein from the cytoplasmic membrane.

Conclusions. SPase functions at the terminal step of the general secretory pathway by releasing translocated proteins from the cytoplasmic membrane at a defined cleavage site. We have provided evidence of an inducible alternative terminal step in *S. aureus* encoded by the *ayrRABC* operon, which under normal conditions is repressed by AyrR but which is derepressed by SPase inhibition. The natural physiological role of the pathway may be to support SPase function at times when elevated secretion is required and the capacity of SPase has been exceeded. Alternatively, it may have evolved to facilitate survival in the presence of an SPase inhibitor. The latter possibility is consistent with the fact that SPase is the only component of the general secretory pathway that is exposed on the outer surface of the cytoplasmic membrane, making it uniquely susceptible to extracellular inhibitors. The evolution of four different families of arylomycins (10) further suggests that that SPase inhibition may have represented a significant selection pressure, as does the existence of multiple, redundant SPases in many Gram-positive bacteria.

Hints as to the mechanism by which AyrABC mediates the release of proteins from their attachment to the cell may be gleaned from an analysis of the individual genes. *ayrB* and *ayrC* encode an ABC transporter. It is possible that the ABC transporter functions as part of the translocation process, perhaps augmenting or even replacing other components of the general secretory pathway. However, bacterial ABC transporters have been identified that extract lipophilic moieties from the cytoplasmic membrane (24), and AyrBC may function after Sec translocation to extract intact preproteins or proteins after intramembrane cleavage. The role of AyrA, which encodes a membrane protein of unknown function, is even less clear, but it is interesting to speculate that it could be a protease responsible for intramembrane cleavage, if one is required, which is consistent with its predicted membrane localization. It is also interesting that *ayrR* homologs in other species appear to control the regulation of proteases (25, 26). A more complete understanding of the pathway awaits detailed analysis of these proteins as well as the signal responsible for derepression of the operon, which is in progress. Undoubtedly, understanding the mechanistic details of this alternate terminal step of the Sec pathway will lead to a greater understanding of secretion and the response to secretion stress.

SUPPLEMENTAL MATERIAL

Supplemental material for this article may be found at http://mbio.asm.org/lookup/suppl/doi:10.1128/mBio.01178-15/-/DCSupplemental.

Figure S1, TIF file, 0.8 MB.
Figure S2, TIF file, 0.1 MB.
Figure S3, TIF file, 0.3 MB.
Table S1, DOC file, 0.01 MB.
Table S2, DOC file, 0.03 MB.
Table S3, DOC file, 0.01 MB.
Table S4, DOC file, 0.01 MB.
Table S5, DOC file, 0.03 MB.

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REFERENCES

1. **Blobel G, Sabatini DD**. 1971. Ribosome-membrane interaction in eukaryotic cells, p 193–195. *In* Manson LA (ed), Biomembranes. Springer, New York, NY.
2. **Milstein C, Brownlee GG, Harrison TM, Mathews MB**. 1972. A possible precursor of immunoglobulin light chains. Nat New Biol **239:**117–120. http://dx.doi.org/10.1038/newbio239117a0.
3. **Du Plessis DJ, Nouwen N, Driessen AJ**. 2011. The Sec translocase. Biochim Biophys Acta **1808:**851–865. http://dx.doi.org/10.1016/j.bbamem.2010.08.016.
4. **Rusch SL, Kendall DA**. 2007. Interactions that drive Sec-dependent bacterial protein transport. Biochemistry **46:**9665–9673. http://dx.doi.org/10.1021/bi7010064.
5. **Driessen AJ, Nouwen N**. 2008. Protein translocation across the bacterial cytoplasmic membrane. Annu Rev Biochem **77:**643–667. http://dx.doi.org/10.1146/annurev.biochem.77.061606.160747.
6. **Schallenberger MA, Niessen S, Shao C, Fowler BJ, Romesberg FE**. 2012. Type I signal peptidase and protein secretion in *Staphylococcus aureus*. J Bacteriol **194:**2677–2686. http://dx.doi.org/10.1128/JB.00064-12.
7. **Schimana J, Gebhardt K, Holtzel A, Schmid DG, Sussmuth R, Muller J, Pukall R, Fiedler HP**. 2002. Arylomycins A and B, new biaryl-bridged lipopeptide antibiotics produced by streptomyces sp. Tü6075. I. Taxonomy, fermentation, isolation and biological activities. J Antibiot (Tokyo) **55:**565–570.
8. **Roberts TC, Smith PA, Cirz RT, Romesberg FE**. 2007. Structural and initial biological analysis of synthetic arylomycin A_2. J Am Chem Soc **129:**15830–15838. http://dx.doi.org/10.1021/ja073340u.
9. **Smith PA, Roberts TC, Romesberg FE**. 2010. Broad spectrum antibiotic

activity of the arylomycin natural products is masked by natural target mutations. Chem Biol **17:**1223–1231. http://dx.doi.org/10.1016/j.chembiol.2010.09.009.
10. **Tan YX, Romesberg FE.** 2012. Latent antibiotics and the potential of the arylomycins for broad-spectrum antibacterial activity. Med Chem Comm **3:**916–925. http://dx.doi.org/10.1039/c2md20043k.
11. **Roberts TC, Schallenberger MA, Liu J, Smith PA, Romesberg FE.** 2011. Initial efforts toward the optimization of arylomycins for antibiotic activity. J Med Chem **54:**4954–4963. http://dx.doi.org/10.1021/jm1016126.
12. **Liu J, Smith PA, Steed DB, Romesberg F.** 2013. Efforts toward broadening the spectrum of arylomycin antibiotic activity. Bioorg Med Chem Lett **23:**5654–5659. http://dx.doi.org/10.1016/j.bmcl.2013.08.026.
13. **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.** 2012. Broadening the spectrum of beta-lactam antibiotics through inhibition of signal peptidase type I. Antimicrob Agents Chemother **56:**4662–4670. http://dx.doi.org/10.1128/AAC.00726-12.
14. **Powers ME, Smith PA, Roberts TC, Fowler BJ, King CC, Trauger SA, Siuzdak G, Romesberg FE.** 2011. Type I signal peptidase and protein secretion in *Staphylococcus epidermidis*. J Bacteriol **193:**340–348. http://dx.doi.org/10.1128/JB.01052-10.
15. **Craney A, Romesberg FE.** 2015. A putative Cro-like repressor contributes to arylomycin resistance in *Staphylococcus aureus*. Antimicrob Agents Chemother **59:**3066–3074. http://dx.doi.org/10.1128/AAC.04597-14.
16. **Huffman JL, Brennan RG.** 2002. Prokaryotic transcription regulators: more than just the helix-turn-helix motif. Curr Opin Struct Biol **12:**98–106. http://dx.doi.org/10.1016/S0959-440X(02)00295-6.
17. **Sarai A, Takeda Y.** 1989. Lambda repressor recognizes the approximately 2-fold symmetric half-operator sequences asymmetrically. Proc Natl Acad Sci U S A **86:**6513–6517. http://dx.doi.org/10.1073/pnas.86.17.6513.
18. **Ptashne M.** 1986. A genetic switch: gene control and phage λ, 3rd ed. Cell Press and Blackwell Scientific Publications, Cambridge, MA.
19. **Mesak LR, Yim G, Davies J.** 2009. Improved lux reporters for use in *Staphylococcus aureus*. Plasmid **61:**182–187. http://dx.doi.org/10.1016/j.plasmid.2009.01.003.
20. **Smith PA, Powers ME, Roberts TC, Romesberg FE.** 2011. In vitro activities of arylomycin natural-product antibiotics against *Staphylococcus epidermidis* and other coagulase-negative staphylococci. Antimicrob Agents Chemother **55:**1130–1134. http://dx.doi.org/10.1128/AAC.01459-10.
21. **Washburn MP, Wolters D, Yates JR III.** 2001. Large-scale analysis of the yeast proteome by multidimensional protein identification technology. Nat Biotechnol **19:**242–247. http://dx.doi.org/10.1038/85686.
22. **Inloes JM, Hsu KL, Dix MM, Viader A, Masuda K, Takei T, Wood MR, Cravatt BF.** 2014. The hereditary spastic paraplegia-related enzyme DDHD2 is a principal brain triglyceride lipase. Proc Natl Acad Sci U S A **111:**14924–14929. http://dx.doi.org/10.1073/pnas.1413706111.
23. **Hyyryläinen HL, Sarvas M, Kontinen VP.** 2005. Transcriptome analysis of the secretion stress response of *Bacillus subtilis*. Appl Microbiol Biotechnol **67:**389–396. http://dx.doi.org/10.1007/s00253-005-1898-1.
24. **Narita S.** 2011. ABC transporters involved in the biogenesis of the outer membrane in gram-negative bacteria. Biosci Biotechnol Biochem **75:**1044–1054. http://dx.doi.org/10.1271/bbb.110115.
25. **Biswas S, Biswas I.** 2014. A conserved streptococcal membrane protein, LsrS, exhibits a receptor-like function for lantibiotics. J Bacteriol **196:**1578–1587. http://dx.doi.org/10.1128/JB.00028-14.
26. **Sun H, Xu Y, Sitkiewicz I, Ma Y, Wang X, Yestrepsky BD, Huang Y, Lapadatescu MC, Larsen MJ, Larsen SD, Musser JM, Ginsburg D.** 2012. Inhibitor of streptokinase gene expression improves survival after group A streptococcus infection in mice. Proc Natl Acad Sci U S A **109:**3469–3474. http://dx.doi.org/10.1073/pnas.1201031109.