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The modulation of *Staphylococcus aureus* mRNA turnover

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Abstract

Staphylococcus aureus is a Gram-positive pathogen capable of causing a wide array of infections owing, in large part, to the coordinated expression of an extensive repertoire of virulence factors. Our laboratory and others have shown that the expression of these factors can occur post-transcriptionally at the level of mRNA turnover and is mediated by ribonucleases, RNA-binding proteins, and regulatory RNA molecules. Moreover, *S. aureus* harbors the ability to alter the stability of its mRNA titers in response to physiological stresses, including antibiotic exposure. Although ongoing studies are attempting to identify the molecular components that modulate *S. aureus* mRNA turnover, innovative approaches to target these essential processes have established a novel group of targets for therapeutic development against staphylococcal infections.

Keywords

drug discovery; mRNA turnover; pathogenesis; ribonuclease; *Staphylococcus aureus*; virulence

Staphylococcus aureus causes more deaths in the USA annually than AIDS [1]. The organism owes its ability to cause disease, in large part, to the coordinated regulation of an expansive repertoire of virulence factors that aid the pathogen in colonizing a host, evading the host immune system, disseminating to secondary sites and adapting to host-associated ‘environmental’ stresses [2]. *S. aureus* gene regulation has historically been considered to occur at the level of transcript synthesis. Indeed, an array of two-component regulatory systems (TCRS) and DNA-binding transcription factors provide *S. aureus* with the ability to directly alter transcript synthesis in response to intra- and extra-cellular cues (Figure 1A) [2–4]. However, steady-state mRNA levels are a function of both RNA synthesis and RNA degradation, and it is becoming increasingly recognized that the modulation of mRNA turnover is an important means of regulating *S. aureus* gene expression [5].

Recent evidence indicates that the organism alters its mRNA degradation properties in response to growth phase [6] and extracellular environmental stresses [7,8], including antibiotic exposure [9,10]. Moreover, changes in mRNA turnover appear to have

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biologically significant outcomes and have been associated with increased antibiotic tolerance [7], adherence to epithelial cells [9] and pathogenesis [11]. This article will focus on what is currently understood regarding *S. aureus* mRNA turnover, focusing on three known functional groups of the mRNA turnover machinery: ribonucleases (RNases), RNA-binding proteins and regulatory RNA molecules.

Ribonucleases

Ribonucleases have been best characterized within the Gram-negative and Gram-positive model organisms, *Escherichia coli* and *Bacillus subtilis*, respectively (reviewed in [12]). In *E. coli*, bulk mRNA degradation is performed by a multiprotein complex, termed the RNA degradosome, consisting primarily of RNase E (*rne*; 5'-end-dependent endoribonuclease), PNPase (*pnpA*; 3'→5' exonucleolytic polynucleotide phosphorylase), RhlB (*rhlB*; DEAD-box RNA helicase B) and enolase (*eno*; glycolytic enzyme) [13–15]. RNase E is believed to be the central component of the *E. coli* degradosome, serving as the scaffold for the assembly of other subunits and catalyzing the initial endoribonucleolytic event during substrate degradation [16,17]. PNPase is believed to degrade resulting transcript fragments in a 3'→5' fashion with RhlB facilitating this process by relieving complex secondary structures [18,19]. The characterization of an equivalent complex in Gram-positive organisms, including *S. aureus*, had been thwarted by the absence of an RNase E amino acid ortholog. However, in 2005 Even *et al.* identified two *B. subtilis* ribonucleases, RNase J1 and J2 (*rnjA/B*), which exhibited RNase E-like 5'-end-dependent endonucleolytic activity and affected global mRNA turnover [20]. Yet, it was not until 2009, when a putative *B. subtilis* degradosome was identified, that the similarities between RNase J1/J2 and RNase E were fully appreciated (Figure 1B). Utilizing the *B. subtilis* model, we recently identified an orthologous degradosome-like complex in *S. aureus* [21]. As shown in Figure 1B, this complex appears to share components with that of both *E. coli* and *B. subtilis* (PNPase and enolase) and *B. subtilis* alone (RNase J1/J2, RNase Y [*rny*; 5'-end-dependent endoribonuclease] and phosphofructokinase [*pfk*; glycolytic enzyme]) [22] and includes the DEAD-box RNA helicase, CshA, which is thought to serve as a functional equivalent to the *E. coli* RhlB [21,23]. Despite these recent advances in defining the Gram-positive mRNA turnover machinery, the mechanism(s) by which these enzymes interact to coordinate mRNA degradation is still unknown. What is known regarding mRNA turnover in *S. aureus* is limited to studies of the individual contributions of enzymes both *in vitro* and *in vivo*. This article will focus on the roles of individual ribonucleases in RNA degradation and their respective contributions to *S. aureus* mRNA turnover based on the three known classes of enzymes: endoribonucleases, exoribonucleases and toxin-mediated ribonucleases.

Endoribonucleases

S. aureus is thought to produce at least seven endoribonucleases, each of which exhibits high sequence similarity to known *B. subtilis* RNases. Of these, RNase III (*rnc*) and RNase P (*rnpAB*) have been characterized to directly affect *S. aureus* mRNA turnover. RNase III catalyzes dsRNA degradation. While its cellular role has historically been limited to that of rRNA processing and maturation, the enzyme was recently described to also play a major role in *S. aureus* virulence factor expression [11,24–26]. The RNA–RNA duplexes formed between the regulatory RNA molecule, RNAIII (described later), and target mRNA transcripts result in a dsRNA template sufficient to initiate RNase III-mediated degradation, the result of which contributes to the tight regulation of virulence factor expression (Figure 1C) [26]. RNase P is an endoribonuclease that has also been associated with *S. aureus* mRNA turnover. The enzyme is a ribozyme consisting of RNA (*rnpB*) and protein (*rnpA*) subunits, and has historically been considered to be involved in tRNA maturation [27]. More recently, the protein component of RNase P, RnpA, was shown to affect the mRNA turnover properties of 24% of the organism's transcripts, suggesting that it contributes to bulk *S.*

aureus RNA degradation [28]. Furthermore, cells with diminished RnpA function exhibited limited proliferation, establishing RnpA as an essential *S. aureus* protein and also suggesting that mRNA turnover itself is an essential process that could be exploited for antibiotic drug discovery. Knowledge regarding the role of additional endoribonucleases in *S. aureus* mRNA turnover is limited. Nonetheless, the *S. aureus* RNases J1, J2 and Y, all members of the *S. aureus* degradosome-like complex, are highly conserved with respect to the orthologous *B. subtilis* enzymes. In preliminary studies, *S. aureus* RNases J1 and Y were shown to degrade rRNA and other RNA substrates *in vitro* with varying efficiencies, but further studies are needed to classify these and other enzymes (RNases HII, HIII and BN) with respect to their ability to process mRNA in a physiologically relevant setting [28]. In studies with *B. subtilis*, RNases J1 and J2 were shown to cleave the leader region of the *thrS* transcript (encoding threonyl-tRNA synthetase) at a site identical to that observed with RNase E in *E. coli* [20]. Moreover, the activity of *B. subtilis* RNases J1 and J2 were dependent on the phosphorylation state of the 5'-end of the transcript in an RNase E-like manner as well. However, unique to RNase J1 is its activity as a 5'→3' exoribonuclease (described later), highlighting the notion that Gram-positive organisms may utilize different enzymatic strategies for mRNA turnover [29]. It should also be noted that the membrane-associated RNase Y was found to be required for virulence in *S. aureus* [30], however it is unclear whether the observed phenotype was due directly to an alteration in mRNA stability [31]. Based on the recent discovery of a putative *S. aureus* degradosome, additional studies characterizing the individual functions of these and other endoribonucleases, as well as the role of enzymatic interactions, are highly anticipated.

Exoribonucleases

Exoribonucleases have been characterized as the 'work horses' of bacterial and eukaryotic mRNA turnover [32]. The *S. aureus* genome encodes for three putative exoribonucleases: PNPase (*pnpA*), RNase R (*rnr*) and YhaM (*cbfI*) (Table 1). Of these, PNPase has been shown to play a role in *S. aureus* mRNA turnover; only 17.6% of all transcripts within $\Delta pnpA$ cells were degraded at 5 min post-transcriptional arrest, whereas approximately 50% of transcripts in wild-type cells were degraded [12]. This was the first study to identify PNPase as a modulator of mRNA turnover in *S. aureus* and also suggested that, because *pnpA* is not an essential gene, other enzymes can compensate for a loss of PNPase activity. Presumably, redundant functions of RNase R and/or YhaM account for the dispensability of PNPase, but this has not yet been established. Nonetheless, RNase R is a 3'→5' hydrolytic exoribonuclease that has been characterized in both *E. coli* and *B. subtilis* to cleave polyadenylated mRNA, tRNA, and rRNA containing significant secondary structure *in vitro* and may exhibit similar functions in *S. aureus* [33,34]. The third *S. aureus* exoribonuclease, YhaM, was shown to exhibit 3'→5' exoribonuclease activity *in vitro* [35], but additional studies are needed to fully assess the role(s) of *S. aureus* exoribonucleases in global mRNA turnover. *S. aureus* also harbors a non-traditional exoribonuclease, RNase J1. Although previously characterized for its endonucleolytic activity, RNase J1 was described to exhibit an unprecedented 5'→3' exoribonuclease activity in *B. subtilis*. This particular direction of degradation, as opposed to the more traditional 3'→5' activity described earlier, is unique to RNase J1 and is hypothesized to contribute to the stability of RNA molecules. Preliminary studies suggest that when stabilizing factors, such as stalled ribosomes or secondary structures, inhibit the access of RNase J1 to the 5'-end of its substrates, downstream regions of the transcript are no longer degraded [29]. While it remains to be seen whether this function is conserved across bacterial species, it seems reasonable to predict that RNase J1 contributes to *S. aureus* mRNA turnover. Collectively, the exoribonucleases constitute a class of RNases into which future studies will provide important insight into the terminal fate of transcripts and subsequent effects on gene expression.

Toxin–antitoxin systems

Toxin–antitoxin systems have recently been shown to play a role in modulating global mRNA turnover during situations that are taxing for cellular growth. During stressful conditions, induced proteases degrade the labile antitoxin, whereas the stable toxin persists within the cell, the result of which is believed to contribute to bacteriostasis and, ultimately, survival until the stress condition subsides [36]. The *E. coli* MazF/PemK family of toxins exhibit endoribonuclease activity both *in vitro* and *in vivo*, and homologs have been identified in *Mycobacterium tuberculosis* [37], *B. subtilis* [38], and more recently, *S. aureus* [39]. *S. aureus* MazF toxin is co-transcribed with its antitoxin counterpart, MazE, as a component of the *sigB* stress-inducible regulon [39]. Fu *et al.* demonstrated that *S. aureus* MazF overproduction results in bacteriostasis [40], suggesting that this particular toxin–antitoxin system may serve as a stop-gap to allow stress adaptation, as opposed to a *bona fide* toxin. Indeed, a cursory assessment of MazF ribonuclease activity indicates that it catalyzes digestion of nonessential genes, such as the virulence factor transcripts *hla* (α -hemolysin) and *spa*. But, the enzyme does not affect mRNA species generated from the essential genes, *recA* (DNA repair) or *gyrB* (DNA gyrase), despite containing similar numbers of predicted cleavage sites as found in *hla*, *spa* and *sigB*. Although future studies are needed to study the role of endogenous MazF *in vivo*, these preliminary studies establish toxin-mediated mRNA turnover as a putative member of the many mechanisms *S. aureus* has developed to cope with stress.

RNA-binding proteins

RNA-binding proteins are an important class of mRNA turnover modulatory factors that directly interact with target transcripts and, in turn, affect their degradation. The function of RNA-binding proteins has been most extensively characterized in *E. coli*. In particular, the *E. coli* host factor I protein (Hfq) is an RNA-binding protein that binds polyadenylated and uracil-rich regions of mRNA transcripts, sequestering regions subject to cleavage by 3'→5' exoribonucleases and RNase E, respectively [41]. In addition to its role as a stabilizing agent, Hfq can catalyze the degradation of target transcripts by facilitating the coupling of mRNAs to regulatory sRNAs and, consequently, catalyzing the cleavage of both RNA species by RNases (Figure 1D) [42]. A second, well-characterized RNA-binding protein, the *E. coli* CsrA, has also been shown to differentially modulate mRNA turnover of target transcripts related to metabolism, biofilm formation and virulence factors [43–46]. Like Hfq, CsrA can destabilize certain RNA species but also stabilizes others. For instance, the association of CsrA with the 5' UTR of the *pgaA* transcript blocks binding of the 30S ribosomal subunit, thus inhibiting translation and destabilizing the transcript [45], whereas CsrA has been implicated in stabilizing *flhDC* (encoding for a transcriptional regulator of flagellum expression), a phenomenon that is thought to contribute to increased motility of the organism [46].

As stated earlier, *S. aureus* mRNA turnover properties change in response to certain stresses; it is likely that RNA-binding proteins are among the *trans*-acting factors that modulate the organism's RNA degradation properties. However, the identity and role of any such proteins are limited. Hfq does not play an obvious role in currently defined *S. aureus* mRNA–sRNA reactions and one early report suggested that a Δhfq strain does not exhibit any recognizable phenotype [47,48]. More recently, Liu *et al.* showed that Hfq is a global regulator that affects the expression of at least 116 genes and affects *S. aureus* pathogenicity [49]. The discrepancy between these studies is presumably owing to differential expression characteristics of Hfq in genetically divergent strains of *S. aureus*. Indeed, Liu *et al.* showed that Hfq was readily expressed in the 8325-4 strain used for their studies, however the RNA-binding protein was not detected in the strains COL and 6390 (which lacked any discernable phenotype in isogenic Δhfq strains) [49]. Regardless, further studies are required to formally

assess what role, if any, *S. aureus* Hfq exhibits as a *trans*-acting factor of mRNA stability. Nonetheless, there is mounting evidence that the organism may harbor additional putative RNA-binding proteins. In a study evaluating the effect of the global regulator, SarA, on mRNA half-lives, Roberts *et al.* noted that 138 mRNA molecules exhibited longer half-lives in wild-type cells than in an isogenic *sarA* deletion strain [6]. The structure of SarA and its ability to bind AT-rich regions of DNA are similar to that of the *E. coli* histone-like proteins HU and H-NS [50]. Interestingly, HU and H-NS have been shown to bind RNA in *E. coli*, including mRNAs thought to be involved with stress adaptation and virulence. Moreover, HU and H-NS have been implicated in the post-transcriptional regulation of gene expression by associating with the 5' UTR region of transcripts that in turn modulate translation [51] and the stability of the targeted mRNAs [52]. Although SarA itself has not yet been shown to bind directly to mRNA transcripts, its ability to affect the half-lives of numerous transcripts, including the virulence factors *cna* (encoding collagen-binding protein) and *spa*, suggests an important role for SarA in mRNA turnover and provides a mechanism by which the factor affects gene expression.

Regulatory RNA molecules

Small, regulatory RNA (sRNA) molecules are perhaps the best characterized class of *S. aureus* mRNA turnover modulatory factors. As the name implies, sRNAs are generally limited in size (50–300 nt), contain no predicted open reading frame and exhibit regulatory functions. Through interactions with small molecule ligands, proteins, and other RNA molecules, sRNAs are thought to modulate a variety of physiologic functions, including mRNA turnover.

The most extensively studied mechanism of action for sRNAs is direct RNA–RNA interactions consisting of two broad classes: *cis*-acting and *trans*-acting sRNAs. *Cis*-acting sRNAs are contained within the transcriptional unit of the gene itself. Meanwhile, *trans*-acting sRNAs often share little spatial relationship to the genes that they regulate and are typically complementary to only small stretches of their targets, allowing a single *trans*-sRNA species to modulate the transcript titers of an array of mRNA target molecules. An exception to the aforementioned characteristics would be antisense RNAs (asRNAs), which are transcribed from the same region of DNA as the strand of the gene they regulate, and typically include significant (75 bp or more) complementarity between the asRNA and its target transcript. Upon forming a sRNA–mRNA duplex, the dsRNA complex is frequently targeted by ribonucleases and consequently represses expression of the target gene(s) [53]. Through these interactions, sRNAs have been implicated in the repression of virulence factors, stress response genes, toxins and metabolic functions [54,55].

Trans-acting *S. aureus* sRNAs

More than 200 putative sRNAs have been identified to date within *S. aureus* [6,7,48,56–59]. Of these, RNAIII is by far the best characterized [60] and was originally identified as a global virulence factor regulatory locus [61]. In laboratory culture conditions, maximal RNAIII expression occurs during late-exponential and stationary-phase growth [62], which correlates with simultaneous repression of *S. aureus* cell surface-associated virulence factors but induction of extracellular virulence factors [3]. While RNAIII includes a short open reading frame (encoding δ -hemolysin) it has long been known that the RNA molecule itself, as opposed to δ -hemolysin, regulates *S. aureus* virulence factor production [60]. Yet, until recently, the mechanism by which RNAIII modulates virulence factor expression was poorly understood. In a series of studies, it was demonstrated that RNAIII is a *trans*-acting sRNA whose regulatory effects are mediated by base pairing with target transcripts, thereby altering their translation and stability [24–26,63,64]. These effects are largely repressive in nature, and primarily involve cell surface-associated virulence factors [3]. For example,

RNAIII can repress protein A expression by binding directly to the 5' terminus of the *spa* mRNA [11]. Formation of this RNA–RNA duplex is sufficient to inhibit translation, but also serves as a substrate for RNase III digestion. Indeed, while *spa* generally exhibits a very short half-life in wild-type *S. aureus* cells, the transcript is stabilized in the absence of RNAIII ($t_{1/2} = \sim 15$ min). In addition to its suppressive role, RNAIII can also directly activate gene expression. For instance, RNAIII binding to α -hemolysin (virulence factor) mRNA exposes the transcript's ribosomal binding site and, consequently, facilitates toxin production [64].

Although RNAIII is the most extensively studied *S. aureus* regulatory RNA, a plethora of additional regulatory RNAs are predicted to exist [65]. Of the confirmed *trans*-acting sRNAs, the *S. aureus* pathogenicity island-related RNA, SprD, is required for virulence and directly binds the *sbi* transcript (encoding an IgG binding protein). However, *sbi* mRNA titers are not changed in the presence of SprD, suggesting that the transcript may not undergo degradation as described with other sRNA–mRNA interactions [66]. Additional studies are needed to formally assess the stability of the *sbi* transcript in the presence and absence of SprD as it is unclear why the dsRNA complex does not initiate RNase III-mediated degradation as is described for other sRNA–mRNA interactions. A third regulatory RNA, RsaE, can also bind directly to its targets, however, the effects of this interaction on the turnover of the targeted transcripts are currently unknown [57,58]. Further studies to empirically assess the regulatory effects of putative *S. aureus* sRNAs are required and will undoubtedly demonstrate that the organism produces additional regulatory RNAs.

Riboswitches

Riboswitches are known for their ability to sense and respond to intracellular metabolites and are found almost exclusively in the 5' UTR regions of RNA molecules, providing a means for directly regulating transcripts immediately downstream. Association with the metabolite typically results in an alteration of the RNA's secondary structure, liberating or sequestering the ribosomal binding site, initiating premature transcript termination or altering the stability of the transcript [67]. In *B. subtilis*, the degradation of the riboswitch-controlled transcripts is initiated by RNase Y and subsequent degradation requires additional components of the *B. subtilis* degradosome, however, this remains uncharacterized in *S. aureus* [29]. Although numerous examples for riboswitch-mediated alteration of transcription and translation exist, it has only recently been recognized that riboswitches are likely to affect mRNA stability. The first evidence for a riboswitch that could modulate mRNA stability originated from a study that used a bioinformatics-based search for conserved *cis*-acting regions of mRNAs [68]. Of those discovered, the putative *glmS* riboswitch contained a unique region that, when bound to its ligand, glucosamine-6-phosphate (GlcN6P), initiated a site-specific cleavage of the 5' leader of the transcript, resulting in the destabilization of the molecule [69]. The cleavage of the 5' UTR region results in a 5'-hydroxyl end and then initiates further degradation of the transcript in an RNase J1-dependent, 5'→3' exonucleolytic manner [70]. This mechanism appears to be conserved in *S. aureus* and is hypothesized to play an important role in the organism's pathogenesis [71]. Although *glmS* is currently the only riboswitch known to directly modulate mRNA turnover in *S. aureus*, additional studies specifically assessing the roles of other known and unknown riboswitches in global mRNA turnover may reveal additional important regulators.

Conclusion

S. aureus mRNA turnover involves a complex and highly coordinated set of processes that are essential for growth and provide the pathogen with a mechanism for post-transcriptionally modulating gene expression. Indeed, comparisons of the mRNA

degradation profiles from cells challenged with physiologic stresses suggest that *S. aureus* can modify its RNA turnover properties, presumably in an effort to regulate gene expression and quickly mount stress responses while conserving cellular resources. Although research characterizing mRNA turnover in *E. coli* and other Gram-negative organisms has provided a wealth of knowledge, little is known regarding mRNA turnover in *S. aureus*. The modulation of virulence factor expression by RNase III and RNase III has served as a paradigm for post-transcriptional regulation since the early 1990s, however, recent evidence supports the notion that mRNA turnover in *S. aureus* is no less complex than better-studied organisms, and likely involves a repertoire of RNases, regulatory RNAs and RNA-binding proteins. The continuing characterization of *S. aureus* RNA degradation machinery is obviously needed and the recent discovery of novel sRNAs that directly affect the organism's pathogenicity establishes hundreds of candidates for further studies into *S. aureus* post-transcriptional gene regulation. A better understanding of this phenomenon will certainly be exciting and provide insight into novel regulatory circuits that may contribute to the fastidious control of *S. aureus* virulence factors and its ability to persist at epidemic proportions.

Future perspective

In an age where *S. aureus* resistance to currently available antibiotics is escalating and the majority of these therapeutics target translation machinery or are later-generation antibiotics [72], the need for new targets for drug discovery is ever growing as the pathogen has reached epidemic proportions in both hospital and community settings [1,73,74]. The essentiality of mRNA turnover in bacteria serves as an enticing target for antimicrobial therapeutic development. In addition to being an essential biological process, mRNA degradation and stability also directly affect virulence factor expression and stress adaptation. Accordingly, the opportunity for targeting mRNA turnover and post-transcriptional gene regulation for novel drug targets is an avenue worth exploring. Initial studies evaluating the inhibition of mRNA turnover as a route for inhibiting cell growth and pathogenesis have been promising. Indeed, small molecules capable of inhibiting the RNA-degrading function of RNase P have been shown to ameliorate *S. aureus* disease [28]. Moreover, these compounds did not affect the growth of the two Gram-negative organisms tested (*E. coli* and *Acinetobacter baumannii*) and exhibited limited toxicity on eukaryotic cells, which suggest a correlation between the efficacy of the small molecules and the amino acid similarity of RNase P across different species [28]. The success of such an approach highlights both the importance of mRNA turnover as well as the potential for RNA turnover machinery as a novel class of antimicrobials. Thus, defining the factors that modulate *S. aureus* mRNA degradation may not only advance our understanding of the mechanisms by which the organism copes with environmental stresses and regulates its repertoire of virulence factors, but may also provide novel strategies for the therapeutic intervention of staphylococcal infections.

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Executive summary

Staphylococcus aureus

- *Staphylococcus aureus* is a Gram-positive opportunistic pathogen capable of causing a wide array of infections. Post-transcriptional control of virulence factor expression occurs at the level of mRNA turnover.

Physiologic role for mRNA turnover

- The degradation of mRNA transcripts is an essential process in both prokaryotes and eukaryotes.
- *S. aureus* globally alters the stability of its mRNA in response to physiologic stresses, including antibiotic exposure. mRNA stability is associated with increased antibiotic tolerance, adherence to epithelial cells and pathogenesis.

Ribonucleases

- Ribonucleases have been shown previously to function as a multienzyme complex termed the degradosome. No such orthologous holoenzyme has yet been identified in *S. aureus*. *S. aureus* contains at least 14 genes with sequence homology to *Bacillus subtilis* RNases. RNase III, RNase P and PNPase have been characterized to affect mRNA turnover in *S. aureus*. Other RNases have limited functional characterization.

RNA-binding proteins

- In *Escherichia coli* and other Gram-negative bacteria the RNA-binding protein, Hfq, modulates mRNA stability by facilitating mRNA–sRNA interactions. Recent reports suggest that the *S. aureus* Hfq does not function in this manner. The DNA-binding protein, SarA, affects mRNA half-lives in *S. aureus*. Its molecular mechanism remains undetermined.

Regulatory RNA molecules

- Over 200 putative regulatory RNA molecules exist in *S. aureus*. One of these, RNAIII, is expressed in a growth phase-dependent manner; its expression represses cell surface-associated and activates secreted extracellular virulence factor production. RNAIII directly interacts with target mRNAs, inhibiting translation and activating degradation by RNase III. The regulatory RNA, SprD, also functions by directly interacting with its target mRNA, *sbi*. Both RNAIII and SprD directly affect the pathogenesis of *S. aureus*.

Future perspective

- The majority of commercially available antibiotics are later generation drugs. Innovative studies identified *S. aureus* mRNA turnover machinery as effective antimicrobial targets both *in vitro* and *in vivo*. Additional studies characterizing mRNA turnover in *S. aureus* may identify additional targets for therapeutic development.

Figure 1. Control of *Staphylococcus aureus* gene expression

(A) The control of *Staphylococcus aureus* gene expression is historically attributed to an array of TCRS that operate in coordination with DNA-binding transcription factors to propagate extra- and intra-cellular signals and ultimately activate (*Act*) or repress (*Rep*) target gene transcript synthesis. (B) Bulk mRNA degradation is thought to be mediated by a holoenzyme complex, termed the degradosome. The *S. aureus* degradosome-like complex consists of at least eight enzymes, including the RNases J1, J2, Y, P and PNPase, the glycolytic enzymes enolase and Pfk, and the DEAD-box RNA helicase, CshA. (C) Regulatory RNAs, in part, modulate gene expression by affecting degradosome and/or ribonuclease functions; *S. aureus* RNAIII can bind target mRNAs, sequester the Shine–Dalgarno sequence and create sRNA–mRNA duplex substrates for RNase III digestion. (D) *Escherichia coli* sRNA–mRNA interactions are facilitated by the RNA-binding protein, Hfq, and often result in destabilization; Hfq can also stabilize target mRNAs by sequestering RNase E recognition sites, preventing degradation of the transcript. The SarA transcriptional regulator was found to influence the global half-lives of transcripts, although the mechanism for this observation remains undetermined. RNase: Ribonuclease; TCRS: Two-component regulatory system.

Table 1*Staphylococcus aureus* ribonucleases

Ribonuclease type	Gene symbol
<i>Endoribonucleases</i>	
RNase III	<i>rnc</i>
RNase BN/Z	SA1335 [†]
RNase HII	<i>mhb</i>
RNase HIII	SA0987 [†]
RNase J1	SA0940 [†]
RNase J2	SA1118 [†]
RNase M5	SA0450 [†]
RNase P	<i>mpaB</i>
RNase Y	SA1129 [†]
<i>Exoribonucleases</i>	
PNPase	<i>pppA</i>
RNase R	<i>rnr</i>
YhaM	<i>yhaM/cbfI</i>
<i>Toxin-mediated ribonuclease</i>	
MazF	<i>mazF</i>

[†] *Staphylococcus aureus* N315 loci of genes homologous to *Bacillus subtilis* RNases.

RNase: Ribonuclease.