Post-transcriptional control of virulence gene expression in *Staphylococcus aureus*

Alexandre Le Scornet, Peter Redder

► To cite this version:

Alexandre Le Scornet, Peter Redder. Post-transcriptional control of virulence gene expression in *Staphylococcus aureus*. *Biochimica et Biophysica Acta - Gene Regulatory Mechanisms*, 2019, 1862, pp.734 - 741. 10.1016/j.bbagr.2018.04.004 . hal-03484705

HAL Id: hal-03484705

<https://hal.science/hal-03484705>

Submitted on 20 Dec 2021

HAL is a multi-disciplinary open access archive for the deposit and dissemination of scientific research documents, whether they are published or not. The documents may come from teaching and research institutions in France or abroad, or from public or private research centers.

L'archive ouverte pluridisciplinaire **HAL**, est destinée au dépôt et à la diffusion de documents scientifiques de niveau recherche, publiés ou non, émanant des établissements d'enseignement et de recherche français ou étrangers, des laboratoires publics ou privés.

Distributed under a Creative Commons Attribution - NonCommercial| 4.0 International License

Post-transcriptional control of virulence gene expression in *Staphylococcus aureus*

Authors:

Alexandre Le Scornet and Peter Redder

LMGM, Centre de Biologie Integrative,

Paul Sabatier University

118, Route de Narbonne

31062 Toulouse.

France

Corresponding author: Peter Redder, peter.redder@univ-tlse3.fr

Abstract

Opportunistic pathogens have to be ready to change life-style whenever the occasion arises, and therefore need to keep tight control over the expression of their virulence factors. Doubly so for commensal bacteria, such as *Staphylococcus aureus*, which should avoid harming their hosts when they are in a state of peaceful co-existence. *S. aureus* carries very few sigma factors to help define the transcriptional programs, but instead uses a plethora of small RNA molecules and RNA-RNA interactions to regulate gene expression post-transcriptionally. The endoribonucleases RNase III and RNase Y contribute to this regulatory diversity, and provide a link to RNA-decay and intra-cellular spatiotemporal control of expression. In this review we describe some of these post-transcriptional mechanisms as well as some of the novel transcriptomic approaches that have been used to find and to study them.

1. Introduction

One of the major discoveries in the field of bacterial gene regulation, was that RNA molecules not only serve as passive intermediates between DNA and protein, but themselves play an important and active role in regulating both translation and transcription [1]. This realisation has led to the search, in recent years often by transcriptomic methods, for RNAs (or sub-sections of RNAs) with potential regulatory function. A number of excellent recent reviews dealt with the RNA-mediated regulatory mechanisms discovered in various bacteria and have outlined some of the methods used to probe/analyse the molecular details of these regulatory events [2–6].

In this review we highlight some particularly interesting examples of post-transcriptional regulation of virulence factors in *Staphylococcus aureus*, as well as describe some of the transcriptomic methods employed to gain a better understanding of these regulatory mechanisms, which until recently have been largely overlooked.

S. aureus is an opportunistic pathogen which benignly colonises the nasopharynx in 20-30% of the human population [7]. Under conditions such as weakened host immune system or direct access to the bloodstream, these same peaceful strains can change to aggressive pathogens, and need a highly organised gene regulatory system to ensure correct transition between the two lifestyles. In contrast to many bacteria, *S. aureus* does not depend on a large array of sigma factors to perform the fine-tuning of cellular programs, and only four sigma factors have been identified, two of them with cryptic expression conditions [8,9]. *S.*

aureus relies instead on a finely tuned homeostasis between transcription regulators, small RNAs, RNA degradation, and intra-molecular RNA-RNA interactions to generate the required regulatory flexibility [2].

One of the most intriguing examples of RNA-mediated post-transcriptional regulation is RNAIII from *S. aureus*, which with its 14 hairpin structures can interact with, and regulate, a variety of RNA targets involved in virulence [2]. Bacterial mRNAs have short half-lives, typically below 3 minutes [10], and the steady-state level of a given RNA is therefore highly dependent on even minor changes in its degradation rate. What determines the degradation rate is unclear for most RNAs in *S. aureus*, but it appears that a “decay-initiating” endonucleolytic cleavage is the rate-limiting step, at least for a sub-set of RNAs. This is the case for about a hundred coding regions of RNA whose half-lives are dependent on the endonuclease RNase Y (see also section 5) [11], as well as several cases in which the formation of an RNA-RNA duplex results in cleavage by the double-stranded RNase III (see also section 4) [2]. However, endonucleolytic cleavage can also be used to differentially regulate genes that are part of the same transcript, by dividing the transcript into two RNA molecules with different decay-rates [12,13].

2. The 5' and 3' untranslated regions

Understanding the molecular details of post-transcriptional regulation, be it via secondary structures or degradation, requires detailed knowledge of the true extent of the RNA molecule examined. Most standard RNA sequencing protocols systematically lose sequence information from the ends of the RNAs. An example is the dUTP-based library preparation for RNA sequencing with Illumina technology (such as TruSeq), where the true 3'-end of the RNA is lost through use of random primers to generate the cDNA, and 5'-end information is lost when RNA fragments generated by RNase H treatment of the RNA-cDNA duplex are used as primers for generating the second DNA strand. However, in the last couple of years a range of novel methods have been developed which enable exact mapping of the RNA ends [14–20].

2.1 Mapping the 5' ends

Mapping of the 5'-ends have been the focus of most transcriptomic analyses, since this will not only reveal any potential hairpins that can sequester the ribosome binding site, or binding sites for small RNAs (see section 3, below), but also pinpoint the transcription start

1 site on the genome (Figure 1). The most commonly used transcriptome-wide 5'-end
2 analyses involve ligating a synthetic RNA oligo to the 5' ends of all RNA in the sample
3 (Figure 1B), followed by high throughput RNA sequencing primed from the synthetic oligo
4 into the sequence of the cellular RNA. *In silico* analysis of the sequencing reads can find the
5 known sequence of the oligo, and the 5'-end of the original RNA can then easily be identified
6 downstream of the oligo sequence (Figure 1G).

7 A key question which often arises when analysing 5' ends, is whether a detected 5'-end is
8 tri-phosphorylated. Tri-phosphorylated nucleosides are present only at the original 5'-ends of
9 transcripts, and therefore indicate bona fide transcription start events (Figure 1A). Once the
10 transcription start site is known, the full extent of the 5'-UTR is revealed, along with putative
11 secondary structures and binding sites for small regulatory RNAs. In addition, once the
12 transcription start site is mapped onto the genome of the organism, the pre-transcriptional
13 regulation signals on the DNA, such as promoters and repressor binding sites, can often be
14 identified.

15 However, it is not trivial to determine whether a detected 5'-end is tri-phosphorylated, since
16 RNA sequencing does not directly reveal the phosphorylation state of the transcript ends. In
17 addition, ligation of an oligo to the 5'-ends (described above; see Figure 1B) can only be
18 performed when the 5'-ends are mono-phosphorylated. Hence, it is necessary treat the RNA
19 enzymatically, with a pyrophosphatase, to convert tri-phosphorylated 5'-ends to mono-
20 phosphates (Figure 1E), thus losing any information about the original phosphorylation state!
21 Note that this problem also besets non-transcriptomic 5'-end mapping methods such as
22 primer extension or 5'-RACE, although the addition of a pyrophosphatase in the latter can
23 help distinguish processed (i.e. mono-phosphorylated) from tri-phosphorylated 5'-ends
24 [21–23].

25 The solution to this problem was the development of differential RNA sequencing (dRNA-
26 seq), in which RNA samples are split into two aliquots, each prepared separately for
27 sequencing in a slightly different manner. One aliquot is digested with a 5' to 3'
28 exoribonuclease, which is specific for mono-phosphorylated 5'-ends and will ignore the tri-
29 phosphorylated RNA species. Both aliquots are then treated with a pyrophosphatase to
30 convert tri-phosphates into mono-phosphates, followed by ligation to an oligo of known
31 sequence. The two preparations are then converted to cDNA and deep-sequenced
32 separately. Most of the transcriptome in the two samples will look highly similar, but careful
33 comparison of the results from the two sequencings, either by inspection [24] or using
34 sophisticated statistical methods [25–27] permits the identification of regions that are
35 relatively enriched in the exoribonuclease-treated sample. Such an enrichment is caused by
36 the exonuclease protection offered by the tri-phosphorylation, leading to a relative over-

1 representation of the 5'-ends of tri-phosphorylated RNA fragments in the exoribonuclease-
2 treated aliquot [15].
3

4 2.2 The 3'-ends, a relatively unexplored field

5
6 Recently, it has been realised that the 3' UTRs of prokaryote RNA can also play regulatory
7 roles. A global survey of 3' UTRs in *S. aureus*, using a combination of RNA sequencing, tiled
8 micro-arrays and *in silico* analyses, found that 3' UTRs are generally much longer than they
9 need to be to simply terminate transcription, suggesting that many 3' UTRs have additional
10 functions [28]. This study furthermore showed that while rho-independent transcription
11 terminators can be predicted *in silico* with reasonably good accuracy, they are often leaky
12 and permit read-through, which further confound global analyses and structure predictions
13 [28,29].

14 One function for the 3' UTR could be the formation of secondary structures to block 3' to 5'
15 exoribonucleases, and thereby increase the half-life of the RNA, and this does indeed
16 appear to be the case for some transcripts [30]. However, a highly intriguing mechanism was
17 discovered for the *S. aureus icaR* transcript, which encodes the repressor of the operon for
18 biosynthesis of exopolysaccharide for biofilm formation. The *icaR* transcript forms a giant
19 hairpin with a duplex composed of the 5' UTR and the 3' UTR, which regulates IcaR
20 translation on two levels. The first is the RNA half-life, which is lowered by the presence of
21 the hairpin, because it serves as a substrate for the double stranded endoribonuclease
22 RNase III (see section 4 below). This in turn leads to lowered levels of *icaR* RNA and of the
23 encoded protein, unless formation of the hairpin is prevented. Secondly, the base-pairing
24 sequesters the RBS, specifically via a UCCCC-motif which is common to many regulatory
25 RNAs, and prevents translation initiation. Thus there is a competition between ribosome
26 binding and formation of the hairpin, presumably resulting in highly efficient repression of
27 IcaR expression and tight control over this key virulence factor [28]. It is possible that many
28 more examples of regulatory 3'-UTRs can be found in *S. aureus*. It is therefore encouraging
29 that a recently developed method, named Term-seq, is capable of transcriptome-wide
30 mapping of the 3'-ends with single-nucleotide precision, which should reveal both potentially
31 regulatory 3'-UTRs and riboswitch activity in *S. aureus* [31].
32
33

3. Regulating virulence with small RNAs

Virulence in *S. aureus* is tightly linked to quorum sensing, which allows the bacterium to shift between a sedentary life in a biofilm and dispersal throughout the host organism. The quorum sensing itself is mediated by the export of a small peptide that is detected by a two-component system, all encoded in the *agr* operon. However, the intra-cellular effector molecule induced by the two-component system is RNAIII, a multifunctional small RNA, which can post-transcriptionally repress certain genes and induce translation of others [1].

3.1 RNAIII and virulence

RNAIII has 14 stem-loops, providing a range of platforms for hybridising to different RNAs with varying affinities. Three of the loops (loops 7, 13 and 14) are rich in cytosine, which enable them to anneal with the ribosome binding sites of many mRNAs (the consensus Shine-Dalgarno sequence in *S. aureus* is AGGAGG)[32]. Boisset and coworkers used an *in silico* approach to find potential targets for stem-loop 13, by computationally fusing the hypothetical 5' UTR of all open reading frames (ORFs) to the stem-loop 13 sequence, then applying an RNA folding algorithm to determine whether strong secondary structure could be formed by each of these *in silico*-constructed RNAs. The strongest predicted interaction was with the SA1000 mRNA, which encodes a cell-surface-anchored fibrinogen-binding protein [33]; the predicted structure would lead to an unfolding of stem-loop 13 and anti-sense binding to the whole ribosome binding region of the SA1000 mRNA. The predicted free energy of this binding is as high as 34.5 kcal/mol, even though two small bulges disrupt an otherwise perfect complementarity [32]. In order to study interaction between SA1000 mRNA and RNAIII, the *in vivo* 5'-end of the SA1000 mRNA was determined (using primer extension), to ensure that the 5'-UTR did indeed contain the sequence needed to form the predicted structure. The structure of the SA1000 mRNA (both bound and unbound) was probed using a range of *in vivo* and *in vitro* methods, and it was found not only that the strong binding by RNAIII completely prevents the ribosome from initiating translation, but that the resulting ~40 bp double-stranded RNA also serves as cleavage site for the double-stranded endoribonuclease RNase III (see section 4 below), ensuring that the mRNA will not be translated once bound by RNAIII [32].

The many loops in RNAIII also allow more complex interactions, involving more than one region of the target mRNA, thus permitting sophisticated fine-tuning of binding affinities. The *coa* mRNA, encoding the blood-clot-promoting protein staphylocoagulase, is bound by loop 13 around the RBS, and by loop 7 further downstream, inside the coding region. Interestingly, while stem 13 is unfolded upon binding to form an imperfect duplex with the

1 *coa* mRNA, stem 7 remains in hairpin form, so that only the loop itself base-pairs with the
2 loop of a hairpin on the mRNA. *In vitro* binding assays with RNAIII deleted for either of the
3 stem-loop 7 and 13 revealed that the primary binding event appears to be stem-loop 13 with
4 the RBS, since binding of loop 7 to the downstream site is diminished if stem-loop 13 is
5 removed from RNAIII [34].

6 Further complexity is added by the ability of RNAIII to function as an activator on certain of
7 the bound mRNAs, in addition to its mRNA repression role. One example is *hla* mRNA,
8 encoding the cytotoxic hemolysin alpha protein, which forms an intra-molecular secondary
9 structure that sequesters the Shine-Dalgarno sequence [35]. Activation by RNAIII is thought
10 to be achieved by formation of a duplex between the 5'-end of RNAIII and an RNA region on
11 the *hla* mRNA which is complementary to the *hla* Shine-Dalgarno sequence. This duplex,
12 which involves stem-loops 1, 2 and 3 of RNAIII, would liberate the Shine-Dalgarno and thus
13 permit translation initiation [35].

14 SA1000, *coa* and *hla* are only three examples of many RNAIII targets (either predicted or
15 experimentally verified). However, the amount of RNAIII is not infinite in the cell, especially in
16 mid-exponential growth phase, where the quorum sensing system has not yet been fully
17 activated (Figure 2). This means that the various target mRNAs must compete for RNAIII
18 binding, especially since many of them, for example SA1000 and *coa* mRNAs, use the same
19 binding site(s) on RNAIII. How this competition affects RNAIII-mediated virulence regulation
20 *in vivo* remains an open question, whose resolution will require detailed studies of the *in vivo*
21 turnover and localisation of the molecules involved.

22 Finally, it is important to note that no protein factor(s) have been identified as necessary for
23 RNAIII interactions with SA1000 and *coa* mRNAs (no data is available for *hla* mRNA), in
24 contrast to *E. coli* where Hfq protein is needed to promote many of the inter-molecular RNA-
25 RNA regulatory events. *S. aureus* does encode an ortholog of Hfq, and although several
26 observations that *hfq* deletion has no impact on RNA-RNA interactions have been reported
27 [32,34,36], it has been suggested that this may be highly strain-dependent [37], raising again
28 the question of how the equilibrium of RNA-RNA interactions is handled in strains where Hfq
29 has no role?

32 3.2 Other small regulatory RNAs in *S. aureus*

33 The importance of RNAIII as a central virulence regulator has led several groups to search
34 for other regulatory RNAs in *S. aureus*, and more than 500 “non-coding” RNAs have now
35 been identified [38], most of them using various forms of RNA sequencing methods [39–43].

1 However, the analysis methods used do not always distinguish between a true small RNA
2 (meaning a separate short transcript) and an unexpectedly long UTR. Further confounding
3 the matter is the possibility that small RNAs can be shortened by RNases and so change
4 their regulatory specificity, and that some small RNAs are the result of processing of a longer
5 mRNA. An attempt to re-find many of these non-coding RNAs using more modern
6 sequencing methods and stricter inclusion criteria, succeeded in re-finding only 159 small
7 RNAs, which nevertheless remains an impressively high number [38].

8 That said, the mere existence of a short RNA molecule does not mean that it has a
9 regulatory function, and does not by itself identify its RNA targets. MAPS (MS2 affinity
10 purification coupled to RNA sequencing) was developed to identify targets of a specific small
11 RNA, with no prior knowledge of its “targetome” [44]. The MAPS technique was adapted for
12 *S. aureus*, and used to identify RNA partners of the small RNA RsaA. Briefly, the 5'-end of
13 RsaA was fused to a sequence that folds into two hairpins recognised by the MS2 coat
14 protein. This permits the specific purification of the tagged RsaA, as well as any RNA
15 molecules that co-purify with it. Deep sequencing of the total purified RNA (compared to a
16 control sample without tagged RsaA) revealed potential partners, with the previously
17 identified target *mgrA* mRNA at the top of the list [45,46]. Further experiments confirmed the
18 interaction between RsaA and several mRNAs, and demonstrated that RsaA is capable of
19 sequestering ribosome binding sites to prevent translation initiation [45].

20 A complementary strategy for demonstrating regulatory *S. aureus* RNA-RNA interactions *in*
21 *vivo*, consists of co-expressing the small RNA together with the potential mRNA target with a
22 translationally fused green fluorescent protein (GFP). The amount of GFP produced can
23 then be quantified, either on agar plates or with a micro-plate reader, and if GFP expression
24 is dependent on the presence of the small RNA, a direct interaction is a strong possibility
25 [47]. While this strategy is not a global survey method, it is designed to enable rapid
26 screening of many targets, since the small RNA and the target RNAs are expressed from
27 separate vectors which can be combined in various ways. This strategy was initially tested
28 with RNAIII and known interacting mRNAs, and was demonstrated to be useful for showing
29 not only repression by small RNAs, but also RNAIII-dependent positive translational
30 regulation of MapW (an extracellular adherence protein) [47,48]. The technique was further
31 used to discover regulation by the prophage-encoded sRNA SprX2, which down-regulates
32 the expression of extracellular complement-binding protein Ecb. It is intriguing that this
33 protein is also down-regulated by RNAIII via a different interaction [47], and that RNAIII
34 appears also to base-pair directly with SprX1, which is almost identical in sequence to
35 SprX2, suggesting a complex web of interactions between small RNAs themselves as well
36 as with mRNAs [49].

4. RNase III

Intramolecular secondary structures in an mRNA, and hybridisation with regulatory sRNAs, can regulate protein expression in several ways. The ribosome binding site (RBS) can be sequestered or released, sometimes upon intervention of a metabolite, but the secondary structures can also serve as targets for RNase III, which specifically cleaves double-stranded RNA. This frequently leads to a rapid degradation of any RNA with a long double-stranded region, permitting small RNAs to initiate decay of mRNAs, provided the two RNAs share sufficient base-pair complementarity [42]. Interestingly, the sites of RNase III cleavage are not always randomly distributed along a double-stranded RNA, but can be directed to cleave at defined sites by base-mismatch bulges in the duplex [42,50].

4.1 RNase III acts on a multitude of targets

An early transcriptome-wide survey of RNase III-associated RNAs was performed by deep sequencing the RNA that co-immunoprecipitated with either the wild-type RNase III (able to bind and cleave the RNA) or active site mutants of RNase III (able only to bind the RNA) [42]. This revealed a number of virulence transcripts that encode either virulence factors or regulators involved in virulence. In addition, many small regulatory RNAs were identified as likely substrates of RNase III [42]. This powerful global approach permitted the identification of potential regions of RNase III cleavage, and additional primer extension and RACE experiments were performed on many RNAs to both confirm cleavage and map the cleavage sites [42].

4.2 RNase III removes the products of leaky and anti-sense transcription

Tight control over transcription is a challenge for any cell, especially for organisms like *S. aureus*, where the high A+T content in the genome (67%) generates a multitude of sites that fortuitously resemble the A+T rich consensus binding sequence for the house-keeping sigma-factor (TTGACA – 17 bp – TATAAT) [18]. Such “stochastic” transcription start sites would be expected to be equally abundant on either DNA strand, and it was therefore curious that very little anti-sense RNA was detected with strand specific RNA sequencing of a wild-type *S. aureus*. However, when an identical RNA sequencing was performed on an RNase III mutant, it was possible to detect large amounts of anti-sense RNA in the sample,

1 indicating that RNase III initiates degradation of the RNA-RNA hybrids between RNA
2 transcribed from the two DNA strands [41]. Such a process can only remove a small fraction
3 of a highly expressed sense RNA (corresponding to a stoichiometric amount of antisense
4 RNA), but will be able to remove trace amounts of transcript generated by a leaky repressed
5 promoter. RNase III is therefore both a vital factor for specific post-transcriptional regulation
6 of transcripts that form duplexes with small RNAs, as well as ensuring the general fidelity of
7 the pre-transcriptional regulation.

9 5. RNase Y

10
11 As seen above for RNase III, many cellular processes have tightly controlled gene
12 expression that is achieved by a precise and specific initiation of RNA decay. However, not
13 all regulatory cleavage events take place on double stranded RNA. A genome-wide survey
14 of *S. aureus* mutants whose disrupted genes had unknown functions but were conserved in
15 other Gram-positive pathogens, identified a gene (dubbed conserved virulence factor A,
16 *cvfA*) required for full haemolysin production on blood-agar plates and virulence of *S. aureus*
17 in silkworms [51]. It was later discovered that *cvfA* encodes RNase Y, a protein with single-
18 stranded endoribonucleolytic activity (independently discovered in *Bacillus subtilis* [52]), and
19 its importance for virulence was further demonstrated with a murine *S. aureus* bacteraemia
20 model [12].

21 5.1 Localization of RNase Y

22 The RNA polymerase holoenzyme is highly conserved among bacteria, but the RNA
23 degradation machinery does not share this conservation. In *E. coli*, the membrane-
24 associated RNase E is the central component of the degradosome, the major RNA
25 degradation complex [53], but RNase E is absent from many bacterial genomes, including *S.*
26 *aureus*. The discovery of RNase Y, a membrane-associated endoribonuclease of firmicutes
27 that interacts with other participants in RNA degradation, led to the hypothesis that RNase Y
28 plays a role similar to that of RNase E in *Escherichia coli* by acting as a scaffold for the
29 formation of a degradosome-like structure [52]. The RNase Ys of *B. subtilis* and *S. aureus*
30 were indeed shown, using bacterial-two-hybrid assay and by tandem affinity purification, to
31 interact directly with the glycolytic enzyme enolase and the DEAD-box RNA helicase CshA
32 [54,55] The latter protein has been proposed to enable the RNA decay machinery to gain
33 access to cleavage sites embedded in secondary structures [52,56].

1 Deletion of the *cshA* gene in *S. aureus* leads to a very poor growth of the mutant at low
2 temperatures and a defective decay of 113 RNAs. Among these is the quorum sensing
3 mRNA *agr*, whose consequent accumulation is presumed to cause the over-production of
4 haemolysins observed in this mutant [55,57]. Surprisingly, removal of the RNase Y
5 membrane anchor results in almost complete reversal of the $\Delta cshA$ -phenotypes.
6 Furthermore this suppressive effect is directly linked to the sub-cellular localization of RNase
7 Y, since the re-anchoring of the anchorless RNase Y (via dimerisation with an enzymatically
8 dead RNase Y) virtually eliminates the rescue of $\Delta cshA$ phenotypes [11]. This phenomenon
9 could be explained by access of an anchor-less RNase Y to RNA molecules that are
10 normally inaccessible when RNase Y is confined to the membrane. This is consistent with
11 preliminary results from our lab that indicate a general shortening of RNA half-lives in the
12 anchorless RNase Y mutant and offer a possible explanation of its suppressive effect on the
13 *cshA* deletion. The sub-cellular localization of RNase Y thus seems to play a significant role
14 in limiting its activity. This raises the question of the localization of RNA molecules inside the
15 cell: is there a sub-population of RNAs localized near the membrane and if so, how is this
16 partition regulated?

17 Proteins destined for export or bound to the membrane frequently carry an N-terminal signal
18 peptide. This peptide is then recognized by the signal recognition particle, which transports
19 the nascent protein, the translating ribosome and the mRNA being translated to the
20 membrane [58]. This active system ensures that exported and membrane bound proteins
21 are correctly addressed to the membrane but as a consequence also transports mRNAs that
22 could be cleaved by the membrane-anchored RNase Y. However, combining data on RNase
23 Y-dependent RNA decay with those generated by a trans-membrane and signal-peptide
24 recognition algorithm showed that for degradation of most membrane-protein-encoding
25 mRNAs, RNase Y-activity is not rate limiting step [59]. It is therefore unlikely that the signal
26 peptide is the major determining factor in RNase Y-dependent mRNA degradation, and the
27 true reason for the membrane localisation of RNase Y is to be sought elsewhere.

28 5.2 RNase Y post-transcriptionally mediates virulence factors

29 Recent experiments have shown that in an RNase Y deletion mutant, many virulence-related
30 ORFs had a longer half-life and a correspondingly increased steady state abundance,
31 although the set of virulence factors affected changes according to the experimental setup
32 and the lineage of *S. aureus* used. This is especially evident for decay of the *agr* quorum-
33 sensing transcript, which is affected differently in three separate studies of RNase Y mutants
34 [11,12,51]. One of the most thoroughly studied examples of the effect of RNA cleavage on
35 virulence is the processing of the *saePQRS* operon transcript by RNase Y. This operon was

1 shown to be involved in the regulation of haemolysins, nucleases and coagulase when
2 exposed to α -defensins or H_2O_2 , which are both produced upon phagocytosis [60]. SaeS
3 acts as a kinase sensor and SaeR as a transcription regulator, forming a two-component
4 system. SaeP and SaeQ have been proposed to assist the Sae system in returning to its
5 pre-activation state by negative feedback [61]. The processing of this operon by RNase Y
6 allows differential expression of the co-transcribed genes, due to a precise RNase Y
7 cleavage between *saeP* and *saeQ* (Figure 3). The cleavage induced by the RNase induces
8 rapid degradation of the upstream RNA fragment and stabilization of the downstream
9 fragment, leading to the prevalence of *saeRS* [13]. Thus, RNA degradation can enable a
10 rapid change in expression profiles making RNase Y an important player in this virulence-
11 regulation network.

12 A better understanding of how RNase Y selects its target would certainly provide more
13 information on its regulatory role in virulence. It is striking that the half-lives of only about a
14 hundred open reading frames are significantly prolonged in an RNase Y mutant, indicating
15 high selectivity [11]. To show whether those RNAs are in fact cleaved by RNase Y and
16 where, Exact Mapping Of Transcriptome Ends (EMOTE) was used. EMOTE is a
17 transcriptome-wide approach based on the ligation of a synthetic RNA oligonucleotide to the
18 5' ends of total RNA, similar to the transcription start site mapping described in section 2
19 (see also Figure 1). However, no pretreatment of the RNA is needed, since RNase Y
20 cleavages leave a 5' mono-phosphate ready for ligation. Comparing EMOTE data on WT
21 and an RNase Y mutant to identify 5'-ends absent from the RNase Y mutant resulted in a list
22 of 99 RNase Y cleavage sites. Bioinformatic analysis of those sites showed that RNase Y
23 preferentially cleaves after a G in an A/U-rich region but that neither condition is an absolute
24 requirement [11]. Furthermore, deletion of 13 nucleotides surrounding the cleavage site of the
25 *sae* operon did not prevent its cleavage by RNase Y, but rather moved the cleavage site
26 exactly 13 nucleotides upstream, leading to the proposal that RNase Y recognizes nearby
27 secondary structures downstream of the cleavage site [13]. Although bioinformatic analyses
28 of the EMOTE data did not reveal a common secondary structure within 50 nucleotides of
29 the RNase Y cleavage sites, Marincola and coworkers identified a sequence element named
30 Rrs (for RNase Y recognition sequence) immediately downstream of the cleavage site which
31 can form a putative secondary structure with its counterpart, located a further ~80
32 nucleotides downstream. RNase Y cleavage was observed whenever the Rrs and its
33 counterpart were present, but none when they were absent, strongly indicating that Rrs and
34 its counterpart forms a structure that guides RNase Y to correctly cleave the *saePQRS*
35 transcript [13].

6. Conclusion

It is not known how many processes are regulated post-transcriptionally by RNA-RNA interactions in *S. aureus*, but new small RNAs and new interactions, demonstrated or potential, are published almost monthly: it appears that virtually every major metabolic or virulence pathway has a small RNA or a regulatory RNA structure somewhere. Is there something about *S. aureus* that makes post-transcriptional regulation preferable? The small number of sigma factors in *S. aureus* must certainly play a role in this, but it is unclear whether the scarcity of sigma factors caused the efficient RNA-mediated regulation to evolve, or whether the efficient RNA-mediated regulation made additional sigma factors redundant. Moreover, commensal bacteria like *S. aureus*, which are adapted to living within the human nasopharynx, experience a changing environment with respects to the presence of other commensals, mucus, host immune system, physical stress etc., but a relatively constant temperature. The formation of RNA-RNA duplexes is sensitive to temperature, especially if they are short, as in the case of the initial interactions between the hairpin-loops of RNAIII and their targets, and a constant temperature makes duplex formation predictable and tractable for fine-tuning - a requirement for precise regulation. Interestingly, many mutants in the RNA decay machinery of *S. aureus* grow very poorly (or not at all) when exposed to temperature changes of as little as 5 to 7°C (i.e at 42°C or 30°C) [57,62,63], perhaps due to misfolding of RNAs. Furthermore, both RNase III and RNase Y appear to base their high cleavage specificity on nearby secondary structure elements [13,42], which suggests that the equilibrium between the three states: structured, single-stranded and actively translated is vital for determining the fate of an RNA molecule. A possible way to study such processes in a transcriptome-wide manner would be to re-analyse the 99 known RNase Y cleavage sites [11] using the knowledge gained from the detailed analyses of the cleavage site in the *sae* transcript [13]. Ideally such an *in silico* analysis could be enhanced immensely both by transcriptome-wide probing of RNA secondary structures, several protocols for which have now been published [64], and by intra-cellular localisation of RNase Y and its RNA targets.

Acknowledgements:

We would like to thank Dr. Dave Lane for reading, commenting on and correcting the English in the manuscript.

Funding:

This work was supported by the Paul Sabatier University, Toulouse and the Centre for Integrative Biology.

Conflict of interest:

The authors have nothing to disclose.

Figure legends:

Figure 1, Preparing RNA for 5'-end sequencing.

RNA is shown in green and DNA in light blue. Known sequence of synthetic oligos is shown as bold lines and unknown sequence as fine lines. A) Four major types of 5'-end has been found in *S. aureus*: original transcripts with tri-phosphorylated 5'-ends, mono-phosphorylated transcripts produced by a pyrophosphohydrolase such as RppH, mono-phosphorylated down-stream products produced by RNases such as RNase Y or RNase III, and non-phosphorylated down-stream products formed by toxin RNase-mediated cleavage or by autocatalyzed. Note that di-phosphorylated and NAD-capped RNA species might also exist, but have yet to be identified. B) To identify the first nucleotide of the 5'-end in mono-phosphorylated RNA, an oligo with known sequence is ligated to all the 5'-ends in the sample of cellular RNA. The RNA is then reverse transcribed to cDNA (for example using a random primer), which will now have the reverse complement of the known oligo sequence at its 3'-end. A primer that hybridises to this known sequence can then be used to perform a sequencing reaction using massive parallel sequencing methods. C) Since mono-phosphorylated RNA species can be readily ligated to the synthetic oligo, it is sometimes convenient to remove all or most of the mono-phosphorylated RNA in the sample when the object of interest is tri-phosphorylated or non-phosphorylated species. Mono-phosphorylated RNA can easily be removed by 5'-exonucleases that are specific for mono-phosphorylated RNA, such as Terminator or XRN1. D) Non-phosphorylated 5'-ends cannot act as substrates for a ligation, and must be mono-phosphorylated by polynucleotide kinase before they can be ligated to a synthetic oligo (see B). E) Tri-phosphorylated 5'-ends cannot act as substrates for a ligation, and must be converted to mono-phosphates by a pyrophosphatase (e.g. Tobacco Acid Pyrophosphatase or RppH) before they can be ligated to a synthetic oligo (see B). F and G) The sequencing reads will consist of a short region of known sequence (shown in bold uppercase and corresponding to a small part of the original oligo that was ligated to the RNA) followed immediately by the first nucleotide of the original RNA 5'-end (shown in lowercase). The oligo sequence (bold uppercase) can be removed *in silico*

from each read, whereupon the sequence original 5'-end of the RNA can be mapped into the genome of the examined organism to identify the exact transcription start site.

Figure 2, Examples of virulence factors regulated by RNAIII.

RNAIII is regulated positively by the quorum sensing *agr* operon. Post-transcriptional regulation is represented by coloured lines, with up-regulation indicated by green arrows and down-regulation by red cross-bars. RNAIII in turn positively regulates MapW and Hla at a post transcriptional level. MapW and Hla prevents leucocyte adhesion and promotes dissemination by lysing host cells, respectively. RNAIII also negatively regulates LytM, Rot, Spa and SarT which respectively promotes: autolysis (via LytM), blood cell toxicity (via Rot) and expression of an IgG binding protein (via SarT). The schematic is simplified for clarity, and the mediators presented have been reported to have many other functions however only the interactions discussed in this review have been included. For a more complete interaction map, please see [3].

Figure 3, RNase Y cleavage of the *sae* operon maintains correct regulation.

The *saePQRS* operon is represented schematically. The RNase Y cleavage site is shown alongside the Rrs counterpart identified by Marincola and coworkers [13]. (A) Processing of the operon by RNase Y leads to rapid degradation of the upstream fragment which in turn leads to the prevalence of SaeRS inside the cell. Phosphorylation of the transcriptional regulator SaeR leads to expression of virulence factors and SaePQ. (B) Absence of RNase Y cleavage results in higher expression of *saeP* which applies a negative feedback on SaeRS, presumably causing loss of SaeRS function.

References

- [1] R.P. Novick, H.F. Ross, S.J. Projan, J. Kornblum, B. Kreiswirth, S. Moghazeh, Synthesis of staphylococcal virulence factors is controlled by a regulatory RNA molecule, EMBO J. 12 (1993) 3967–3975.
- [2] B. Felden, P. Boulloc, Regulatory RNAs in bacteria: From identification to function, Methods San Diego Calif. 117 (2017) 1–2. doi:10.1016/j.ymeth.2017.03.018.
- [3] P. Fechter, I. Caldelari, E. Lioliou, P. Romby, Novel aspects of RNA regulation in Staphylococcus aureus, FEBS Lett. 588 (2014) 2523–2529. doi:10.1016/j.febslet.2014.05.037.
- [4] E.G.H. Wagner, P. Romby, Small RNAs in bacteria and archaea: who they are, what they do, and how they do it, Adv. Genet. 90 (2015) 133–208. doi:10.1016/bs.adgen.2015.05.001.
- [5] D. Bronesky, Z. Wu, S. Marzi, P. Walter, T. Geissmann, K. Moreau, F. Vandenesch, I. Caldelari, P. Romby, Staphylococcus aureus RNAIII and Its Regulon Link Quorum Sensing, Stress Responses, Metabolic Adaptation, and Regulation of Virulence Gene

- Expression, *Annu. Rev. Microbiol.* 70 (2016) 299–316. doi:10.1146/annurev-micro-102215-095708.
- [6] S. Durand, A. Tomasini, F. Braun, C. Condon, P. Romby, sRNA and mRNA turnover in Gram-positive bacteria, *FEMS Microbiol. Rev.* 39 (2015) 316–330. doi:10.1093/femsre/fuv007.
- [7] H.F.L. Wertheim, D.C. Melles, M.C. Vos, W. van Leeuwen, A. van Belkum, H.A. Verbrugh, J.L. Nouwen, The role of nasal carriage in *Staphylococcus aureus* infections, *Lancet Infect. Dis.* 5 (2005) 751–762. doi:10.1016/S1473-3099(05)70295-4.
- [8] K. Morikawa, Y. Inose, H. Okamura, A. Maruyama, H. Hayashi, K. Takeyasu, T. Ohta, A new staphylococcal sigma factor in the conserved gene cassette: functional significance and implication for the evolutionary processes, *Genes Cells Devoted Mol. Cell. Mech.* 8 (2003) 699–712.
- [9] L.N. Shaw, C. Lindholm, T.K. Prajsnar, H.K. Miller, M.C. Brown, E. Golonka, G.C. Stewart, A. Tarkowski, J. Potempa, Identification and characterization of sigma, a novel component of the *Staphylococcus aureus* stress and virulence responses, *PloS One.* 3 (2008) e3844. doi:10.1371/journal.pone.0003844.
- [10] K.L. Anderson, C.M. Roux, M.W. Olson, T.T. Luong, C.Y. Lee, R. Olson, P.M. Dunman, Characterizing the effects of inorganic acid and alkaline shock on the *Staphylococcus aureus* transcriptome and messenger RNA turnover, *FEMS Immunol. Med. Microbiol.* 60 (2010) 208–250. doi:10.1111/j.1574-695X.2010.00736.x.
- [11] V. Khemici, J. Prados, P. Linder, P. Redder, Decay-Initiating Endoribonucleolytic Cleavage by RNase Y Is Kept under Tight Control via Sequence Preference and Sub-cellular Localisation, *PLoS Genet.* 11 (2015) e1005577. doi:10.1371/journal.pgen.1005577.
- [12] G. Marincola, T. Schäfer, J. Behler, J. Bernhardt, K. Ohlsen, C. Goerke, C. Wolz, RNase Y of *Staphylococcus aureus* and its role in the activation of virulence genes, *Mol. Microbiol.* 85 (2012) 817–832. doi:10.1111/j.1365-2958.2012.08144.x.
- [13] G. Marincola, C. Wolz, Downstream element determines RNase Y cleavage of the saePQRS operon in *Staphylococcus aureus*, *Nucleic Acids Res.* (2017). doi:10.1093/nar/gkx296.
- [14] J.M. Schifano, I.O. Vvedenskaya, J.G. Knoblauch, M. Ouyang, B.E. Nickels, N.A. Woychik, An RNA-seq method for defining endoribonuclease cleavage specificity identifies dual rRNA substrates for toxin MazF-mt3, *Nat. Commun.* 5 (2014) 3538. doi:10.1038/ncomms4538.
- [15] C.M. Sharma, J. Vogel, Differential RNA-seq: the approach behind and the biological insight gained, *Curr. Opin. Microbiol.* 19 (2014) 97–105. doi:10.1016/j.mib.2014.06.010.
- [16] V. Pelechano, W. Wei, P. Jakob, L.M. Steinmetz, Genome-wide identification of transcript start and end sites by transcript isoform sequencing, *Nat. Protoc.* 9 (2014) 1740–1759. doi:10.1038/nprot.2014.121.
- [17] P. Redder, Using EMOTE to map the exact 5'-ends of processed RNA on a transcriptome-wide scale, *Methods Mol. Biol. Clifton NJ.* 1259 (2015) 69–85. doi:10.1007/978-1-4939-2214-7_5.
- [18] J. Prados, P. Linder, P. Redder, TSS-EMOTE, a refined protocol for a more complete and less biased global mapping of transcription start sites in bacterial pathogens, *BMC Genomics.* 17 (2016) 849. doi:10.1186/s12864-016-3211-3.
- [19] C.L. Kirkpatrick, D. Martins, P. Redder, A. Frandi, J. Mignolet, J.B. Chapalay, M. Chambon, G. Turcatti, P.H. Viollier, Growth control switch by a DNA-damage-inducible toxin–antitoxin system in *Caulobacter crescentus*, *Nat. Microbiol.* (2016) 16008.
- [20] A. Fouquier d'Hérouel, F. Wessner, D. Halpern, J. Ly-Vu, S.P. Kennedy, P. Serror, E. Aurell, F. Repoila, A simple and efficient method to search for selected primary transcripts: non-coding and antisense RNAs in the human pathogen *Enterococcus faecalis*, *Nucleic Acids Res.* 39 (2011) e46. doi:10.1093/nar/gkr012.
- [21] J. Vogel, V. Bartels, T.H. Tang, G. Churakov, J.G. Slagter-Jäger, A. Hüttenhofer, E.G.H. Wagner, RNomics in *Escherichia coli* detects new sRNA species and indicates parallel transcriptional output in bacteria, *Nucleic Acids Res.* 31 (2003) 6435–6443.

- [22] L. Argaman, R. Hershberg, J. Vogel, G. Bejerano, E.G. Wagner, H. Margalit, S. Altuvia, Novel small RNA-encoding genes in the intergenic regions of *Escherichia coli*, *Curr. Biol. CB.* 11 (2001) 941–950.
- [23] B.A. Bensing, B.J. Meyer, G.M. Dunny, Sensitive detection of bacterial transcription initiation sites and differentiation from RNA processing sites in the pheromone-induced plasmid transfer system of *Enterococcus faecalis*, *Proc. Natl. Acad. Sci. U. S. A.* 93 (1996) 7794–7799.
- [24] C.M. Sharma, S. Hoffmann, F. Darfeuille, J. Reignier, S. Findeiss, A. Sittka, S. Chabas, K. Reiche, J. Hackermüller, R. Reinhardt, P.F. Stadler, J. Vogel, The primary transcriptome of the major human pathogen *Helicobacter pylori*, *Nature.* 464 (2010) 250–255. doi:10.1038/nature08756.
- [25] F. Amman, M.T. Wolfinger, R. Lorenz, I.L. Hofacker, P.F. Stadler, S. Findeiß, TSSAR: TSS annotation regime for dRNA-seq data, *BMC Bioinformatics.* 15 (2014) 89. doi:10.1186/1471-2105-15-89.
- [26] H. Jorjani, M. Zavolan, TSSer: an automated method to identify transcription start sites in prokaryotic genomes from differential RNA sequencing data, *Bioinforma. Oxf. Engl.* 30 (2014) 971–974. doi:10.1093/bioinformatics/btt752.
- [27] G. Dugar, A. Herbig, K.U. Förstner, N. Heidrich, R. Reinhardt, K. Nieselt, C.M. Sharma, High-resolution transcriptome maps reveal strain-specific regulatory features of multiple *Campylobacter jejuni* isolates, *PLoS Genet.* 9 (2013) e1003495. doi:10.1371/journal.pgen.1003495.
- [28] I. Ruiz de los Mozos, M. Vergara-Irigaray, V. Segura, M. Villanueva, N. Bitarte, M. Saramago, S. Domingues, C.M. Arraiano, P. Fechter, P. Romby, J. Valle, C. Solano, I. Lasa, A. Toledo-Arana, Base pairing interaction between 5'- and 3'-UTRs controls *icaR* mRNA translation in *Staphylococcus aureus*, *PLoS Genet.* 9 (2013) e1004001. doi:10.1371/journal.pgen.1004001.
- [29] U. Mäder, P. Nicolas, M. Depke, J. Pané-Farré, M. Debarbouille, M.M. van der Kooi-Pol, C. Guérin, S. Dérozier, A. Hiron, H. Jarmer, A. Leduc, S. Michalik, E. Reilman, M. Schaffer, F. Schmidt, P. Bessièrès, P. Noirot, M. Hecker, T. Msadek, U. Völker, J.M. van Dijl, *Staphylococcus aureus* Transcriptome Architecture: From Laboratory to Infection-Mimicking Conditions, *PLoS Genet.* 12 (2016) e1005962. doi:10.1371/journal.pgen.1005962.
- [30] V. Khemici, A.J. Carpousis, The RNA degradosome and poly(A) polymerase of *Escherichia coli* are required in vivo for the degradation of small mRNA decay intermediates containing REP-stabilizers, *Mol. Microbiol.* 51 (2004) 777–790.
- [31] D. Dar, M. Shamir, J.R. Mellin, M. Koutero, N. Stern-Ginossar, P. Cossart, R. Sorek, Term-seq reveals abundant ribo-regulation of antibiotics resistance in bacteria, *Science.* 352 (2016) aad9822. doi:10.1126/science.aad9822.
- [32] S. Boisset, T. Geissmann, E. Huntzinger, P. Fechter, N. Bendridi, M. Possedko, C. Chevalier, A.C. Helfer, Y. Benito, A. Jacquier, C. Gaspin, F. Vandenesch, P. Romby, *Staphylococcus aureus* RNAIII coordinately represses the synthesis of virulence factors and the transcription regulator Rot by an antisense mechanism, *Genes Dev.* 21 (2007) 1353–1366. doi:10.1101/gad.423507.
- [33] X. Liang, C. Yu, J. Sun, H. Liu, C. Landwehr, D. Holmes, Y. Ji, Inactivation of a two-component signal transduction system, SaeRS, eliminates adherence and attenuates virulence of *Staphylococcus aureus*, *Infect. Immun.* 74 (2006) 4655–4665. doi:10.1128/IAI.00322-06.
- [34] C. Chevalier, S. Boisset, C. Romilly, B. Masquida, P. Fechter, T. Geissmann, F. Vandenesch, P. Romby, *Staphylococcus aureus* RNAIII binds to two distant regions of *coa* mRNA to arrest translation and promote mRNA degradation, *PLoS Pathog.* 6 (2010) e1000809. doi:10.1371/journal.ppat.1000809.
- [35] E. Morfeldt, D. Taylor, A. von Gabain, S. Arvidson, Activation of alpha-toxin translation in *Staphylococcus aureus* by the trans-encoded antisense RNA, RNAIII, *EMBO J.* 14 (1995) 4569–4577.

- [36] C. Bohn, C. Rigoulay, P. Bouloc, No detectable effect of RNA-binding protein Hfq absence in *Staphylococcus aureus*, *BMC Microbiol.* 7 (2007) 10. doi:10.1186/1471-2180-7-10.
- [37] Y. Liu, N. Wu, J. Dong, Y. Gao, X. Zhang, C. Mu, N. Shao, G. Yang, Hfq is a global regulator that controls the pathogenicity of *Staphylococcus aureus*, *PloS One.* 5 (2010). doi:10.1371/journal.pone.0013069.
- [38] M. Sassi, Y. Augagneur, T. Mauro, L. Ivain, S. Chabelskaya, M. Hallier, O. Sallou, B. Felden, SRD: a *Staphylococcus* regulatory RNA database, *RNA N. Y. N.* 21 (2015) 1005–1017. doi:10.1261/rna.049346.114.
- [39] M. Beaume, D. Hernandez, L. Farinelli, C. Deluen, P. Linder, C. Gaspin, P. Romby, J. Schrenzel, P. Francois, Cartography of methicillin-resistant *S. aureus* transcripts: detection, orientation and temporal expression during growth phase and stress conditions, *PloS One.* 5 (2010) e10725. doi:10.1371/journal.pone.0010725.
- [40] C. Bohn, C. Rigoulay, S. Chabelskaya, C.M. Sharma, A. Marchais, P. Skorski, E. Borezée-Durant, R. Barbet, E. Jacquet, A. Jacq, D. Gautheret, B. Felden, J. Vogel, P. Bouloc, Experimental discovery of small RNAs in *Staphylococcus aureus* reveals a riboregulator of central metabolism, *Nucleic Acids Res.* 38 (2010) 6620–6636. doi:10.1093/nar/gkq462.
- [41] I. Lasa, A. Toledo-Arana, A. Dobin, M. Villanueva, I.R. de los Mozos, M. Vergara-Irigaray, V. Segura, D. Fagegaltier, J.R. Penadés, J. Valle, C. Solano, T.R. Gingeras, Genome-wide antisense transcription drives mRNA processing in bacteria, *Proc. Natl. Acad. Sci. U. S. A.* 108 (2011) 20172–20177. doi:10.1073/pnas.1113521108.
- [42] E. Lioliou, C.M. Sharma, I. Caldelari, A.-C. Helfer, P. Fechter, F. Vandenesch, J. Vogel, P. Romby, Global regulatory functions of the *Staphylococcus aureus* endoribonuclease III in gene expression, *PLoS Genet.* 8 (2012) e1002782. doi:10.1371/journal.pgen.1002782.
- [43] B.P. Howden, M. Beaume, P.F. Harrison, D. Hernandez, J. Schrenzel, T. Seemann, P. Francois, T.P. Stinear, Analysis of the small RNA transcriptional response in multidrug-resistant *Staphylococcus aureus* after antimicrobial exposure, *Antimicrob. Agents Chemother.* 57 (2013) 3864–3874. doi:10.1128/AAC.00263-13.
- [44] D. Lalaouna, E. Massé, Identification of sRNA interacting with a transcript of interest using MS2-affinity purification coupled with RNA sequencing (MAPS) technology, *Genomics Data.* 5 (2015) 136–138. doi:10.1016/j.gdata.2015.05.033.
- [45] A. Tomasini, K. Moreau, J. Chicher, T. Geissmann, F. Vandenesch, P. Romby, S. Marzi, I. Caldelari, The RNA targetome of *Staphylococcus aureus* non-coding RNA RsaA: impact on cell surface properties and defense mechanisms, *Nucleic Acids Res.* 45 (2017) 6746–6760. doi:10.1093/nar/gkx219.
- [46] C. Romilly, C. Lays, A. Tomasini, I. Caldelari, Y. Benito, P. Hamman, T. Geissmann, S. Boisset, P. Romby, F. Vandenesch, A non-coding RNA promotes bacterial persistence and decreases virulence by regulating a regulator in *Staphylococcus aureus*, *PLoS Pathog.* 10 (2014) e1003979. doi:10.1371/journal.ppat.1003979.
- [47] L. Ivain, V. Bordeau, A. Eyraud, M. Hallier, S. Dreano, P. Tattevin, B. Felden, S. Chabelskaya, An in vivo reporter assay for sRNA-directed gene control in Gram-positive bacteria: identifying a novel sRNA target in *Staphylococcus aureus*, *Nucleic Acids Res.* 45 (2017) 4994–5007. doi:10.1093/nar/gkx190.
- [48] Y. Liu, C. Mu, X. Ying, W. Li, N. Wu, J. Dong, Y. Gao, N. Shao, M. Fan, G. Yang, RNAIII activates map expression by forming an RNA-RNA complex in *Staphylococcus aureus*, *FEBS Lett.* 585 (2011) 899–905. doi:10.1016/j.febslet.2011.02.021.
- [49] M. Kathirvel, H. Buchad, M. Nair, Enhancement of the pathogenicity of *Staphylococcus aureus* strain Newman by a small noncoding RNA SprX1, *Med. Microbiol. Immunol. (Berl.).* 205 (2016) 563–574. doi:10.1007/s00430-016-0467-9.
- [50] C. Romilly, C. Chevalier, S. Marzi, B. Masquida, T. Geissmann, F. Vandenesch, E. Westhof, P. Romby, Loop-loop interactions involved in antisense regulation are processed by the endoribonuclease III in *Staphylococcus aureus*, *RNA Biol.* 9 (2012) 1461–1472. doi:10.4161/rna.22710.

- [51] C. Kaito, K. Kurokawa, Y. Matsumoto, Y. Terao, S. Kawabata, S. Hamada, K. Sekimizu, Silkworm pathogenic bacteria infection model for identification of novel virulence genes, *Mol. Microbiol.* 56 (2005) 934–944. doi:10.1111/j.1365-2958.2005.04596.x.
- [52] K. Shahbadian, A. Jamalli, L. Zig, H. Putzer, RNase Y, a novel endoribonuclease, initiates riboswitch turnover in *Bacillus subtilis*, *EMBO J.* 28 (2009) 3523–3533. doi:10.1038/emboj.2009.283.
- [53] G.A. Mackie, RNase E: at the interface of bacterial RNA processing and decay, *Nat. Rev. Microbiol.* 11 (2013) 45–57. doi:10.1038/nrmicro2930.
- [54] C.M. Roux, J.P. DeMuth, P.M. Dunman, Characterization of components of the *Staphylococcus aureus* mRNA degradosome holoenzyme-like complex, *J. Bacteriol.* 193 (2011) 5520–5526. doi:10.1128/JB.05485-11.
- [55] C. Giraud, S. Hausmann, S. Lemeille, J. Prados, P. Redder, P. Linder, The C-terminal region of the RNA helicase CshA is required for the interaction with the degradosome and turnover of bulk RNA in the opportunistic pathogen *Staphylococcus aureus*, *RNA Biol.* 12 (2015) 658–674. doi:10.1080/15476286.2015.1035505.
- [56] M. Lehnik-Habrink, H. Pförtner, L. Rempeters, N. Pietack, C. Herzberg, J. Stülke, The RNA degradosome in *Bacillus subtilis*: identification of CshA as the major RNA helicase in the multiprotein complex, *Mol. Microbiol.* 77 (2010) 958–971. doi:10.1111/j.1365-2958.2010.07264.x.
- [57] S. Oun, P. Redder, J.-P. Didier, P. François, A.-R. Corvaglia, E. Buttazzoni, C. Giraud, M. Girard, J. Schrenzel, P. Linder, The CshA DEAD-box RNA helicase is important for quorum sensing control in *Staphylococcus aureus*, *RNA Biol.* 10 (2013) 157–165. doi:10.4161/rna.22899.
- [58] M.M. Elvekrog, P. Walter, Dynamics of co-translational protein targeting, *Curr. Opin. Chem. Biol.* 29 (2015) 79–86. doi:10.1016/j.cbpa.2015.09.016.
- [59] P. Redder, How does sub-cellular localization affect the fate of bacterial mRNA?, *Curr. Genet.* 62 (2016) 687–690. doi:10.1007/s00294-016-0587-1.
- [60] T. Geiger, C. Goerke, M. Mainiero, D. Kraus, C. Wolz, The virulence regulator Sae of *Staphylococcus aureus*: promoter activities and response to phagocytosis-related signals, *J. Bacteriol.* 190 (2008) 3419–3428. doi:10.1128/JB.01927-07.
- [61] D.-W. Jeong, H. Cho, H. Lee, C. Li, J. Garza, M. Fried, T. Bae, Identification of the P3 promoter and distinct roles of the two promoters of the SaeRS two-component system in *Staphylococcus aureus*, *J. Bacteriol.* 193 (2011) 4672–4684. doi:10.1128/JB.00353-11.
- [62] P. Linder, S. Lemeille, P. Redder, Transcriptome-wide analyses of 5'-ends in RNase J mutants of a gram-positive pathogen reveal a role in RNA maturation, regulation and degradation, *PLoS Genet.* 10 (2014) e1004207. doi:10.1371/journal.pgen.1004207.
- [63] J. Armitano, P. Redder, V.A. Guimarães, P. Linder, An Essential Factor for High Mg²⁺ Tolerance of *Staphylococcus aureus*, *Front. Microbiol.* 7 (2016) 1888. doi:10.3389/fmicb.2016.01888.
- [64] C. Del Campo, Z. Ignatova, Probing dimensionality beyond the linear sequence of mRNA, *Curr. Genet.* 62 (2016) 331–334. doi:10.1007/s00294-015-0551-5.

A **B** **C**

Mono-phosphorylated RNAs removed
 with 5' mono-phosphate specific
 exonuclease (ex. XRN1)

D

Non-phosphorylated 5'-end
 (generated by RNase-cleavage)
 5' HO —————

E **F** **G**

(A)

RNase Y cut
(wt)

(B)

No RNase Y cut
(RNase Y mutant)