

## MINIREVIEW

# tRNAs taking charge

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\*Corresponding author: Department of Biochemistry and Molecular Biology, Rutgers University, Robert Wood Johnson Medical School, Piscataway, NJ 08854, USA. Tel: +732-235-4534; Fax: +732-235-5223; E-mail: [nancy.woychik@rutgers.edu](mailto:nancy.woychik@rutgers.edu)<sup>†</sup>Present address: Regeneron Pharmaceuticals, 777 Old Saw Mill River Rd, Tarrytown, NY 10591.**One sentence summary:** Recent studies on the emergence of tRNAs as targets for stress inducible bacterial toxin-antitoxin systems are reviewed; these findings are compared to tRNA cleavage in human stress responses and disease.

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## ABSTRACT

Most bacterial toxins derived from chromosomally encoded toxin-antitoxin (TA) systems that have been studied to date appear to protect cells from relatively short pulses of stress by triggering a reversible state of growth arrest. In contrast to many bacterial toxins that are produced as defense mechanisms and secreted from their hosts, TA toxins exert their protective effect from within the cell that produces them. TA toxin-mediated growth arrest is most frequently achieved through their ability to selectively cleave RNA species that participate in protein synthesis. Until very recently, it was thought that the primary conduit for toxin-mediated translation inhibition was cleavage of a single class of RNA, mRNA, thus depleting transcripts and precluding production of essential proteins. This minireview focuses on how the development and implementation of a specialized RNA-seq method to study *Mycobacterium tuberculosis* TA systems enabled the identification of unexpected RNA targets for toxins, i.e. a handful of tRNAs that are cleaved into tRNA halves. Our result brings to light a new perspective on how these toxins may act in this pathogen and uncovers a striking parallel to signature features of the eukaryotic stress response.

**Keywords:** toxin; antitoxin; VapC; translation inhibition; angiogenin; Rny1

## INTRODUCTION

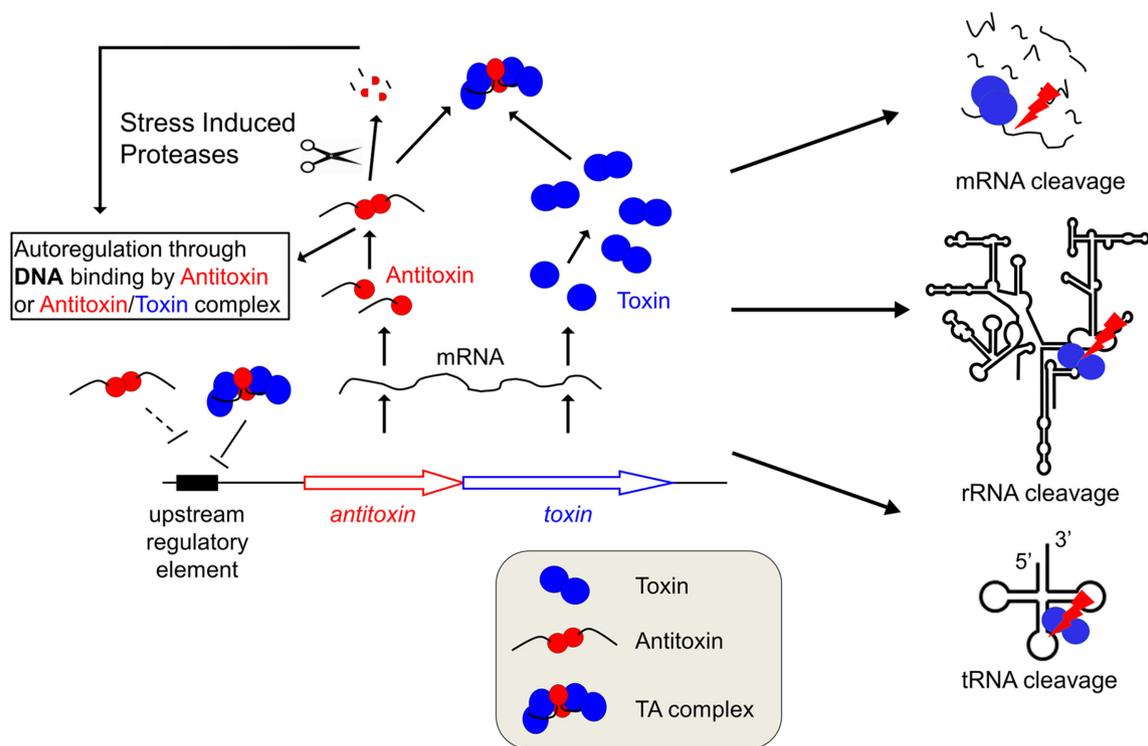
Bacterial toxin-antitoxin (TA) systems comprise adjacent genes encoding a cognate antitoxin and toxin protein. When harbored in the chromosomes of free-living bacteria, the majority of TA systems appear to coordinately enhance stress survival in their host cell. This is achieved through transcriptional regulation of TA operon expression coupled with posttranslational regulation of antitoxin stability. Stress conditions favor degradation of the antitoxin, resulting in free, active toxin (summarized in Fig. 1). Stress survival is most often imparted through growth arrest after inhibition of translation by an RNA-cleaving toxin. Because most of these endoribonuclease toxins had been shown to cleave mRNAs at specific three-, five- or seven-base recognition sequences using conventional methods (Zhang et al. 2003,

2004, 2005; Zhu et al. 2008, 2009; Rothenbacher et al. 2012; Yamaguchi et al. 2012), it was widely believed that mRNAs were the sole target of these TA toxins (Fig. 1). However, our group and others have identified additional RNA targets of TA toxins (Fig. 1), namely rRNA (Vesper et al. 2011; Winther and Gerdes 2011; Moll and Engelberg-Kulka 2012; Schifano et al. 2013, 2016; Winther et al. 2013; Schifano and Woychik 2014) and tRNA (Winther and Gerdes 2011; Cruz et al. 2015). These findings suggest that, in general, the spectrum of RNAs that collaborate in TA system-mediated stress survival is more varied than originally proposed.

TA systems appear to also facilitate stress survival in the pathogen *Mycobacterium tuberculosis*. Stress survival is instrumental for the life cycle of this pathogen, and confounds efforts to treat and eradicate tuberculosis. While tuberculosis still

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**Figure 1.** Summary of RNA-cleaving TA systems. TA systems are composed of two adjacent genes that are cotranscribed and then translated into a stable toxin protein and a labile antitoxin. Under normal conditions, the toxin and antitoxin proteins can form a stable complex in which the toxin is inactive. This complex, or free antitoxin, can bind to an operator upstream of the toxin and antitoxin genes in order to repress their transcription. Under stress conditions, free active toxin accumulates, cleaves RNA, resulting in bacterial growth arrest. Sustained toxin activity results in cell death. Early studies identified several toxins with activity against mRNA. Thus, mRNA has most often been implicated as a toxin target; however, rRNA and tRNA are recently characterized targets of bacterial TA toxins.

causes an estimated 1 million deaths/year worldwide, approximately 90% of those infected with *M. tuberculosis*—comprising approximately one-third of the world's population—have latent tuberculosis (Dye *et al.* 1999). Individuals with latent tuberculosis harbor *M. tuberculosis* within granulomas in the lung, created by the immune response to limit growth to a non-replicating persistent state and sequester the infection. However, granulomas serve as a survival niche for the pathogen, because latent infection can revert to active tuberculosis, especially among the immune compromised.

A better understanding of the players in *M. tuberculosis* growth control is critical to develop antimicrobial therapies that are effective against latent tuberculosis. Since the general function of TA systems is to impart reversible growth inhibition, they are strongly implicated as the molecular switches that enable *M. tuberculosis* to slow or stop replication and become dormant. Consistent with a role in stress survival, several of the 65 annotated TA systems (Table 1) in the *M. tuberculosis* genome are induced during stresses relevant to the pathobiology of this organism: heat shock (Stewart *et al.* 2002), hypoxia (Rustad *et al.* 2008; Ramage, Connolly and Cox 2009), DNA damage (Rand *et al.* 2003), nutrient starvation (Betts *et al.* 2002), macrophage infection (Cappelli *et al.* 2006; Korch, Contreras and Clark-Curtiss 2009; Ramage, Connolly and Cox 2009) and antibiotic treatment (Provvedi *et al.* 2009; Singh, Barry and Boshoff 2010). Also, RNA-seq of nutrient-starved *M. tuberculosis* revealed that 75% of its TA systems were upregulated to some degree with 25% upregulated 2-fold or higher (Cortes *et al.* 2013).

This brief minireview highlights our recent finding that a toxin derived from a VapBC TA system harbored by *M. tuberculosis* exhibits an activity unique among characterized

*M. tuberculosis* TA toxins. We demonstrated that this toxin, VapC-mt4 (Rv0595c, also known as VapC4), is an isoacceptor-specific tRNase (Cruz *et al.* 2015). VapC-mt4 specifically recognizes and cleaves only 3 of the 45 total tRNAs in *M. tuberculosis* at a functionally essential site—the anticodon stem-loop—generating stable tRNA halves (Cruz *et al.* 2015). We also integrate our findings into the field overall, discuss the ramifications of this activity in *M. tuberculosis* cells and examine the striking parallels to the basic features of the eukaryotic stress response.

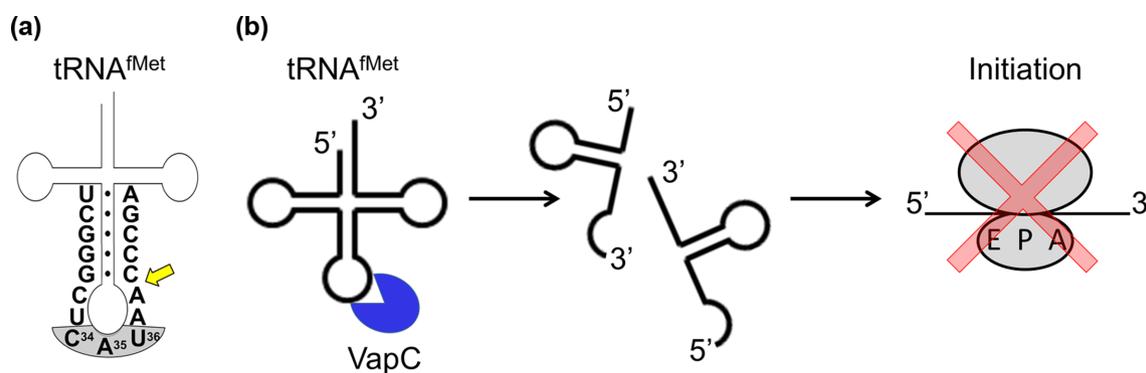
## GENERAL FEATURES OF VapC TOXINS

VapBC TA systems are abundant in bacterial pathogens. In fact, >40% of the approximately 700 TA modules identified in 126 complete genomes of free-living bacteria were VapBC TA family members (Pandey and Gerdes 2005). The multiple copies of VapBC TA systems (though none are identical) are associated with increased virulence, thus they were designated Vap, for virulence associated protein (Katz, Strugnell and Rood 1992). *Mycobacterium tuberculosis* harbors the highest number of VapBC TA systems among free-living bacteria, with an estimated 50 members in its genome (Ramage, Connolly and Cox 2009; Ahidjo *et al.* 2011). All 50 *M. tuberculosis* VapC toxins exhibit structure and sequence similarity and possess a PIN (PilT N-terminal) domain. PIN domains are approximately 130 amino acids in length and contain a quartet of acidic residues and a fifth invariant serine or threonine residue that coordinate divalent cation(s) and possess nuclease activity (Arcus, Rainey and Turner 2005; Arcus *et al.* 2011).

Although the presence of the PIN domain provided some clues to VapC toxin function—each of the characterized VapC

**Table 1.** Toxin-antitoxin systems in *M. tuberculosis*.

No.	TA family	Toxin	Mechanism of toxicity	References
50	VapBC	VapC	Translation; tRNA and rRNA cleavage	Ramage, Connolly and Cox (2009); Ahidjo et al. (2011); Winther et al. (2013); Sala, Bordes and Genevaux. (2014); Cruz et al. (2015)
9	MazEF	MazF	Translation; mRNA and rRNA cleavage	Pandey and Gerdes (2005); Schifano et al. (2013); Schifano et al. (2014); Tiwari et al. (2015)
3	RelBE	RelE	Translation; mRNA cleavage at ribosomal A-site	Korch, Contreras and Clark-Curtiss (2009); Singh, Barry and Boshoff (2010); Yang et al. (2010); Miallau et al. (2013); Korch et al. (2015)
2	ParDE	ParE	Not determined; inhibition of DNA gyrase for ParE from plasmid RK2 and <i>Vibrio cholerae</i> chromosome	Gupta (2009); Ramage, Connolly and Cox (2009); Yuan et al. (2010)
1	HigBA	HigB	Translation; mRNA and tmRNA cleavage by associating with ribosome	Gupta (2009); Fivian-Hughes and Davis (2010); Schuessler et al. (2013)



**Figure 2.** Cleavage of tRNA<sup>Met</sup> by VapC inhibits protein synthesis. (A) The VapC proteins from *Salmonella enterica* serovar LT2 and *Shigella flexneri* 2a virulence plasmid pMYSH6000 were found to cleave the initiator tRNA<sup>Met</sup> within its anticodon stem-loop (yellow arrow). (B) Cleavage of tRNA<sup>Met</sup> by VapC results in the generation of tRNA halves and inhibition of protein synthesis.

toxins are endoribonucleases—their primary RNA targets vary. The *Shigella/Salmonella* (Winther and Gerdes 2011) and *Leptospira interrogans* (Lopes et al. 2014) VapC toxins attack an extremely pivotal player in protein synthesis, tRNA<sup>Met</sup>, and inactivate it through cleavage at a single site in the anticodon stem-loop (Fig. 2). *Mycobacterium smegmatis* VapC cleaves synthetic ssRNAs at a short consensus sequence with some dependence on secondary structure, as well as some mRNAs involved in glycerol uptake and metabolism (McKenzie et al. 2012). Among the 50 *M. tuberculosis* VapC toxins, only two have been characterized in detail. Our laboratory first reported that *M. tuberculosis* VapC-mt4 cleaves at ACGC or AC(A/U)GC sequences in RNA (Sharp et al. 2012), before we were able to pinpoint selected tRNA isoacceptors as targets (Cruz et al. 2015). The Gerdes laboratory reported that VapC20 (VapC-mt20 using our convention) cleaves at the highly conserved sarcin-ricin loop of 23S rRNA (Winther et al. 2013). Thus, although each VapC targets RNA for cleavage, knowing the function of one VapC toxin does not enable prediction of the targets for the family as a whole. Given that there are 50 of these toxins, the ramifications of differential activity among them are provocative and may shed light on the molecular underpinnings of latent tuberculosis.

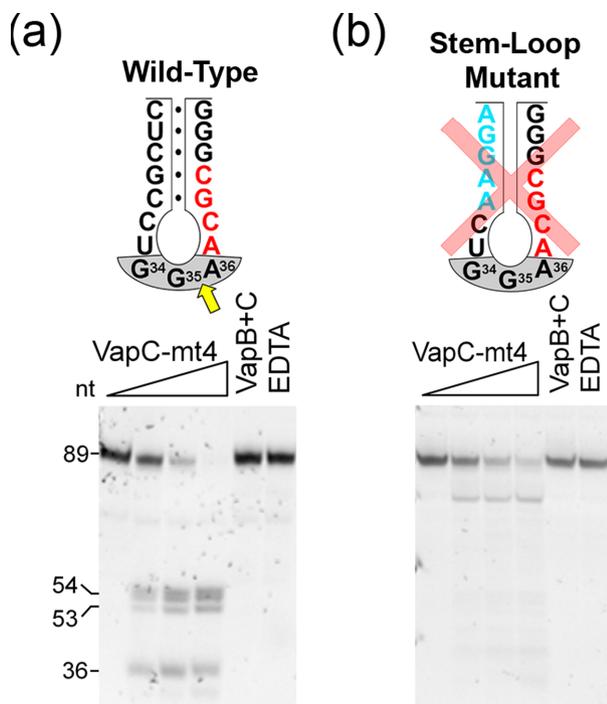
### 5' RNA-Seq TO IDENTIFY RNA TARGETS FOR VapC AND OTHER TA TOXINS

The three VapC-mt4 tRNA targets—tRNA<sup>Ala2</sup>, tRNA<sup>Ser26</sup> and tRNA<sup>Ser24</sup>—were identified using a genome-scale approach, a

specialized RNA-seq method, 5' RNA-seq, that we designed specifically for endoribonuclease toxins (Schifano et al. 2014; Cruz et al. 2015). Conventional bacterial RNA-seq uses massively parallel sequencing technology to sequence the cDNA derived from cellular RNA and is usually intended to survey the entire transcriptome. In contrast, 5' RNA-seq is designed for differential detection of one or more subpopulations of RNA depending on the modification present at the 5' end of the transcript. RNAs in bacterial cells have one of three 5' end modifications: 5' triphosphate (mRNAs), 5' monophosphate (rRNAs and tRNAs) or 5'-hydroxyl (non-coding RNA intermediates and products cleaved by certain endoribonucleolytic toxins such as VapC). 5' RNA-seq enables global analysis of specific populations of RNA transcripts based on their 5' end; i.e. only those transcripts carrying a 5'-PPP, only those transcripts carrying a 5'-P or only those transcripts carrying a 5'-OH (Goldman et al. 2011; Vvedenskaya et al. 2012). Therefore, this method enabled us to specifically detect 5' ends of RNA transcripts that were produced after VapC toxin cleavage (carrying a 5'-OH).

### VapC-mt4 REQUIRES A PRECISE SEQUENCE AND STRUCTURE FOR CLEAVAGE

In contrast to the only other characterized *M. tuberculosis* VapC toxin, VapC-mt20 (which cleaves 23S rRNA at the sarcin-ricin loop; Winther et al. 2013), VapC-mt4 specifically targeted just 3 of the 45 total *M. tuberculosis* tRNAs (tRNA<sup>Ala2</sup>, tRNA<sup>Ser26</sup> and tRNA<sup>Ser24</sup>). Each of these tRNAs contains an ACGC or ACUGC



**Figure 3.** VapC-mt4 requires a consensus sequence within a proper structural context. Cleavage assays comparing wild-type VapC-mt4 cleavage of *M. tuberculosis* tRNA<sup>Ser24</sup> (A) to a mutant that retains the consensus sequence but removes all base pairing in the stem (B). Mutated bases (blue), cleavage site (yellow arrow), consensus sequence (red), anticodon (gray shaded), base-pairing represented as black dots (•). *In vitro* synthesized mutant and wild-type tRNA<sup>Ser24</sup> were incubated with increasing amounts of VapC-mt4 (ratios of toxin to RNA were 0:1, 1.25:1, 2.5:1 and 5:1). Control reactions on the right contained the highest concentration of VapC-mt4 preincubated with VapB antitoxin or EDTA before addition of the respective tRNAs. Reactions were incubated at 37°C for 3 h. Sizes of full length and cleaved tRNA products on the left. Convention when numbering tRNA bases dictates that the anticodon is numbered bases 34–36. However, for the actual base numbers for the anticodon are 35–37.

cleavage consensus sequence (Sharp et al. 2012), indicating that sequence recognition is required for VapC-mt4 cleavage. However, sequence alone cannot account for the selectivity since 13 of the 45 total tRNAs in *M. tuberculosis* also contain ACGC or ACUGC sequences. VapC-mt4 must also require a specific tertiary fold or recognition of determinants in the sugar-phosphate backbone. Indeed, cleavage efficiency was dependent on an intact anticodon stem-loop; cleavage was precluded upon removal of tRNA secondary structure even though the ACGC sequence was intact (Cruz et al. 2015) (Fig. 3A and B).

### STEM LOOPS AS A COMMON DENOMINATOR FOR ALL VapC TARGETS

Even though VapC toxins do not target the same RNAs, each appears to exhibit a preference for stem-loop structures. 23S rRNA cleavage at the sarcin–ricin loop by VapC-mt20 appears to be more dependent on recognition of the stem-loop structure of the sarcin–ricin loop (Fig. 4A) over sequence alone; although the sequences of each bases 5' and 3' of the cleavage site do also matter (Winther et al. 2013). Cleavage of tRNA<sup>fMet</sup> (Fig. 2) occurs in the anticodon stem-loop (Winther and Gerdes 2011; Lopes et al. 2014). Although VapC-mt4 does require the presence of the ACGC or ACUGC consensus sequence, it cuts all three *M. tuber-*

*culosis* tRNA targets precisely within the anticodon loop of tRNA (Fig. 4B–D). These studies, in combination with those from the Arcus group (McKenzie et al. 2012), revealed that the VapC toxin family is distinct from the majority of TA system toxins that possess endoribonuclease activity (e.g. RelE, MazF, HlgB, YafQ) because its members are all predicted to require both sequence and structure elements for target recognition.

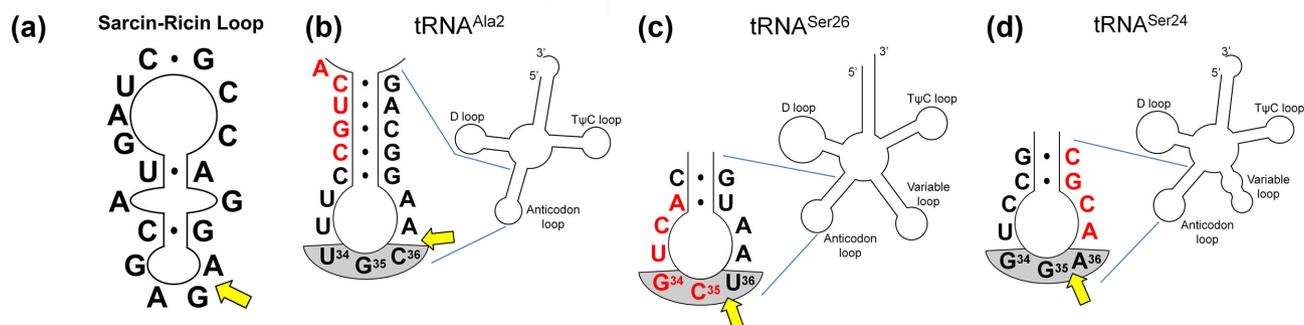
### VapC TOXINS, COLICINS AND OTHER BACTERIAL tRNASES: A COMMON MEANS TO DIFFERENT ENDS

Colicins are bacteriocins produced by strains of *Escherichia coli* to kill nearby cells and reduce competition for resources (Cascales et al. 2007). Colicins are divided into two categories based on their mode of cytotoxicity, pore forming or enzymatic. Enzymatic colicins share mechanistic similarity to VapC toxins because they possess nuclease activity. Two members of this group, colicin D and E5, are tRNases. Death from colicins D and E5 is a consequence of their cleavage of tRNA at a single site within the anticodon stem-loop. Colicin D cleaves all four tRNA<sup>Arg</sup> isoacceptors (Tomita et al. 2000); colicin E5 cleaves tRNA<sup>His</sup> tRNA<sup>Tyr</sup> tRNA<sup>Asn</sup> tRNA<sup>Asp</sup> (Ogawa et al. 1999). In contrast to colicins, which are defensively secreted from the cell, the *E. coli* PrrC endoribonuclease initiates cell suicide of its host cell by cleaving tRNA<sup>Lys</sup> in response to bacteriophage T4 infection (Amitsur, Levitz and Kaufmann 1987; Kaufmann 2000; Meineke and Shuman 2012).

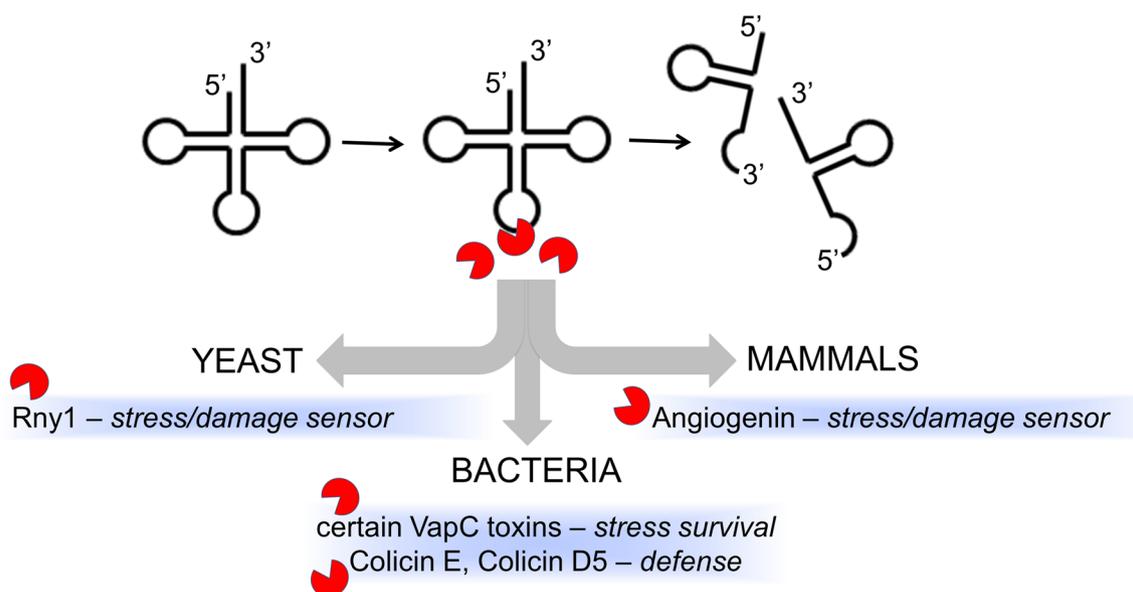
Therefore, in contrast to the growth regulating (and ostensibly reversible) properties of VapC toxins due to carefully orchestrated association with its cognate antitoxin, colicins D, E5 and PrrC are engineered to kill. Death occurs in neighboring cells that take up colicins D and E5 because these cells do not carry the cognate immunity proteins produced by the colicin-secreting attacking cells (Cascales et al. 2007). PrrC-mediated cell death is more subversive. The Stp T4 phage protein alters the association of the ‘antitoxin’ EcoprrI with PrrC, enabling release of free PrrC and unchecked cleavage of lysine tRNA (Amitsur, Levitz and Kaufmann 1987; Kaufmann 2000; Meineke and Shuman 2012).

### MIRROR, MIRROR: tRNA CLEAVAGE IN BOTH EUKARYOTIC AND BACTERIAL STRESS RESPONSES

The generation of tRNA halves upon cleavage at the tRNA anticodon stem-loop and smaller fragments derived from these halves are hallmarks of the eukaryotic stress response (reviewed in Thompson and Parker 2009a; Phizicky and Hopper 2010; Gebetsberger and Polacek 2013). tRNA cleavage in response to a diverse array of stresses has been observed in a wide range of organisms. In the pathogenic protozoa *Trypanosoma cruzi*, tRNA halves are more prevalent upon nutritional stress and associated with specific cytoplasmic granules (Garcia-Silva et al. 2010). Other stresses can also induce tRNA cleavage in eukaryotes. Oxidative stress results in the generation of tRNA fragments in *Saccharomyces cerevisiae*, *Arabidopsis* and human cell lines (Thompson et al. 2008). Nitrogen starvation, methionine starvation, heat shock and nearing lag phase growth also result in increased production of tRNA halves in *S. cerevisiae* (Thompson et al. 2008). In mammalian cells it is known that heat shock, hypothermia, hypotonic stress and hypoxia also led to the accumulation of tRNA halves (Fu et al. 2009; Saikia et al. 2012). Note that cleavage of tRNA is not a general response to all stresses. For



**Figure 4.** VapC proteins target stem-loop structures in rRNA or tRNA. (A) VapC-mt20 (VapC20) targets the conserved sarcin-ricin loop (SRL) of 23S rRNA. VapC-mt4 cleaves three tRNAs from *M. tuberculosis*: tRNA<sup>Ala2</sup> (B), tRNA<sup>Ser26</sup> and (C) tRNA<sup>Ser24</sup>. Yellow arrows indicate the position of cleavage by VapC-mt4. tRNA anticodons are shaded in gray and the VapC-mt4 consensus is indicated in red. Note that in tRNA<sup>Ser26</sup> (C) and tRNA<sup>Ser24</sup> (D) the actual base numbers for the anticodon are 35–37.



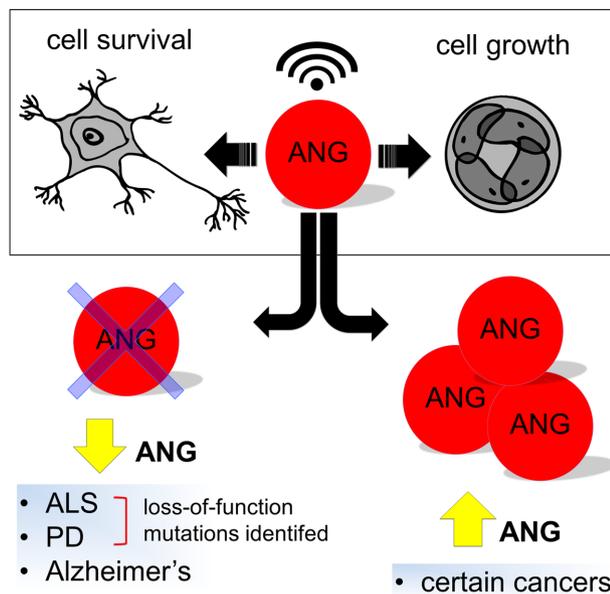
**Figure 5.** tRNA halves are generated in bacteria and eukaryotes. Proteins with known tRNase activity and their general cellular functions are illustrated.

example, tRNA cleavage is not detected in  $\gamma$ -irradiated, caffeine-treated or etoposide-treated human cells (Thompson *et al.* 2008; Fu *et al.* 2009; Yamasaki *et al.* 2009) or in response to UV irradiation, amino acid or glucose starvation in yeast cells (Thompson *et al.* 2008).

There are two eukaryotic RNases associated with stress-linked generation of tRNA halves, *S. cerevisiae* RNase Rny1p and mammalian angiogenin. Rny1p, a member of the RNase T2 family, cleaves tRNAs within their anticodon stem-loop (Thompson and Parker 2009b). Angiogenin, so named because it was originally found to stimulate angiogenesis, is a member of the RNase A family. Both angiogenin and Rny1p have been proposed to act as sensors of cell damage (Thompson and Parker 2009b). In the absence of stress, Rny1p and angiogenin are sequestered in their respective organelles (secreted or in the vacuole for Rny1p; nucleus/nucleolus for angiogenin) and only released to the cytoplasm upon stress. Thus, angiogenin has been proposed to have opposing roles—either promotion of cell growth or promotion of cell survival—depending on its location in the cell (reviewed

in Li and Hu 2012; Saikia and Hatzoglou 2015). To promote cell growth and proliferation, angiogenin translocates to the nucleus/nucleolus where it stimulates rRNA transcription. To promote survival when subjected to certain types of stress, angiogenin accumulates in the cytoplasm and modulates cleavage of tRNA to generate tRNA halves. Although these tRNA halves inhibit most translation, an alternate stress survival mode of translation involving cap-independent internal ribosome entry site initiation is unaffected, resulting in reprogramming of translation (Yamasaki *et al.* 2009; Ivanov *et al.* 2011). Transfection of 5' tRNA halves alone into cultured cells can also inhibit translation and promote stress granule assembly (Emara *et al.* 2010).

Stress generated tRNA halves or other tRNA-derived fragments studied in a variety of eukaryotes, including humans, have been implicated in a range of important cellular processes, including translational control, apoptosis and RNAi regulation (reviewed in Gebetsberger and Polacek 2013; Raina and Ibbá 2014). Therefore, although the signaling pathways and cellular endpoints are clearly distinct, the general features of the stress



**Figure 6.** Angiogenin can influence mammalian cell growth and survival. Angiogenin (ANG) acts as a stress/damage sensor that can influence cell growth (as demonstrated with certain cancers) or cell survival (as demonstrated with neurons). Angiogenin is upregulated in various cancers and downregulated in the neurodegenerative diseases shown (ALS, amyotrophic lateral sclerosis; PD, Parkinson's disease). The illustration only reflects two of the major processes influenced by angiogenin in this emerging field, not all of its known effects.

response are reflected in both the activity of a toxin from the bacterial pathogen *M. tuberculosis* and eukaryotic tRNases responsible for production of stable tRNA halves and other fragments (Fig. 5).

### tRNA HALVES, tRNA-DERIVED FRAGMENTS AND DISEASE

There are many intriguing associations between a variety of human diseases and the stable tRNA halves and/or smaller fragments generated by angiogenin (reviewed in Anderson and Ivanov 2014; Kirchner and Ignatova 2015; Saikia and Hatzoglou 2015) (Fig. 6). Angiogenin is important for neuron survival. To date, downregulation of angiogenin is associated with three neurodegenerative diseases—amyotrophic lateral sclerosis (ALS, Lou Gehrig's disease; Komar and Hatzoglou 2011), Parkinson's disease (PD; Komar and Hatzoglou 2011) and Alzheimer's disease (Kim and Kim do 2012). Mutant forms of angiogenin with reduced RNase activity are implicated in the pathogenesis of ALS. Interestingly, some of these angiogenin mutants associated with ALS were also found in patients with PD (van Es et al. 2011). Consistent with the connection between angiogenin mutants to these two neurodegenerative diseases, addition of recombinant angiogenin to cultured neurons is protective against hypoxia (Sebastia et al. 2009). Angiogenin also increases lifespan and motoneuron survival in an ALS mouse model (Kieran et al. 2008).

Angiogenin is overexpressed in nearly all types of cancer (Li and Hu 2012). However, the significance of this observation is unclear. Genome-scale methods to survey small RNAs are just beginning to track tRNA halves and fragments in certain cancers. For example, deep sequencing of the transcriptomes of prostate cancer patients revealed an increase in tRNA fragments in prostate cancer cells (Martens-Uzunova et al. 2012); yet

tRNA-derived fragments are downregulated in B-cell lymphoma (Maute et al. 2013).

### OUTLOOK

Since only two of the estimated 50 VapC toxins from *M. tuberculosis* have been studied in detail, we have only scratched the surface. VapC-mt4 and VapC-mt20 (VapC20) target different RNAs, tRNA and 23S rRNA sarcin-ricin loop (Winther et al. 2013), respectively. Therefore, it is unlikely that the remaining members will be functionally redundant. Instead, each VapC may cleave unique substrates or sites within a given target, thus allowing a defined molecular response to one or more stresses. Given that *M. tuberculosis* cells can remain dormant yet viable for many years in latent tuberculosis in a granuloma, the resulting activity of each toxin may impart some degree of growth downregulation that is then released before cells reach the point of no return. Thus, through activation of a shifting array of VapC toxins in a calibrated, asynchronous manner, *M. tuberculosis* may be able to limit growth while never reaching the point of cell death.

*M. tuberculosis* cells enlist VapC-mt4 toxin for cleavage of a subset of tRNAs to tRNA halves as vehicles for growth regulation. This finding reveals a glimpse into the intricacies of TA toxin selectivity and the power of this toxin activity in determining the fate of a cell. It also brings to light common themes between the stress response in eukaryotes and bacterial pathogens, adding another layer to the daunting diversity of growth modulators that target a finite set of switches within essential molecular machines. Since VapC toxins rely on both sequence and structure determinants for recognition of their target RNAs, they are endowed with exquisite selectivity. Our findings with tRNA cleaving VapC-mt4 portend the same recognition properties for tRNases in humans and other eukaryotes with pivotal roles in a spectrum of essential processes influencing health and disease.

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**Conflict of interest.** None declared.

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