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RNA Decay: a Novel Therapeutic Target in Bacteria

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Abstract

The need for novel antibiotics is greater now than perhaps any time since the pre-antibiotic era. Indeed, the recent collapse of most pharmaceutical antibacterial groups, combined with the emergence of hypervirulent and pan-antibiotic-resistant bacteria have, in effect, created a “perfect storm” that has severely compromised infection treatment options and led to dramatic increases in the incidence and severity of bacterial infections. Simply put, it is imperative that we develop new classes of antibiotics for the therapeutic intervention of bacterial infections. In that regard, RNA degradation is an essential biological process that has not been exploited for antibiotic development. Herein we discuss the factors that govern bacterial RNA degradation, highlight members of this machinery that represent attractive antimicrobial drug development targets and describe the use of high-throughput screening as a means of developing antimicrobials that target these enzymes. Such agents would represent first-in-class antibiotics that would be less apt to inactivation by currently encountered enzymatic antibiotic-resistance determinants.

Introduction

Infectious diseases are the second-leading cause of death worldwide[1]. Despite this, there has been a mass exodus of pharmaceutical antimicrobial discovery programs, leaving a void in the drug pipeline that, without intervention, will inevitably result in a healthcare crisis. Indeed, the Infectious Diseases Society of America recently warned of antibiotic-resistant ESKAPE bacterial pathogens (*Enterococcus faecium*, *Staphylococcus aureus*, *Klebsiella pneumoniae*, *Acinetobacter baumannii*, *Pseudomonas aeruginosa*, and *Enterobacter* species) and the desperate need for new agents to treat these insidious organisms[2]. Most current antibiotics are derivatives of molecules discovered over 50 years ago and are losing their foothold as effective means of treating infections due to the emergence of antibiotic resistance[3]. Simply put, bacterial antibiotic resistance is outpacing new drug development making it imperative to expand antibiotic drug development to other essential cellular processes in order to create novel agents for the therapeutic intervention of current and emerging antibiotic-resistant bacteria[4]. RNA turnover is one such essential biological process that is rich in antimicrobial targets but has not been exploited for antibiotic drug discovery. Accordingly, this review is intended to bring to light the fundamental differences in RNA turnover between host and bacterial pathogen, distinguish those ribonucleases (RNases) that are attractive antibacterial targets, and provide methods to take advantage of these targets for drug development with the ultimate goal of expanding our antibiotic arsenal.

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mRNA Turnover: Pathogen and Host

Many currently available antibiotics target essential pathways involved in cell wall synthesis, folate metabolism, protein translation, RNA transcription, or DNA replication[3]. These antibiotics are engineered to exert broad antimicrobial activity against an array of bacterial pathogens by targeting essential prokaryotic enzymes within the aforementioned pathways without causing off-target toxic effects toward human counterparts. In that regard, a simple comparison of the physiological characteristics of messenger RNA illustrates wholesale differences between the host and pathogen. For instance, bacteria couple transcription and translation and their mRNA is degraded rapidly (average half-life of ≤ 2.0 min), does not bear a 5' 7-methylguanosine (m^7G) cap, and is rarely 3' polyadenylated. Mammalian cells diverge from their prokaryotic ancestors in that they compartmentalize their RNA metabolic steps and their mRNA has a longer half life (minutes to days), is 5' m^7G capped, and is polyadenylated at the 3' terminus[5]. Therefore, it is not surprising that the molecular machinery that governs bacterial and eukaryotic mRNA degradation differs, and consequently these differences could be exploited for antibiotic drug discovery. As a prerequisite to this approach, one must first appreciate the basic similarities and differences in transcript turnover between the host and pathogen, the RNases involved, and their properties, which are briefly described below. For a more comprehensive report of RNA degradation in these two kingdoms, please refer to several recent excellent reviews[6–11].

The major initiator of bacterial mRNA decay is considered to be a multi-protein complex termed the degradosome. This complex is best characterized in the Gram-negative model organism, *Escherichia coli*, and consists of at least four subunits: RNA helicase B (RhlB), enolase, polynucleotide phosphorylase (PNPase), and RNase E (Figure 1A)[12]. RNase E is the central component of the *E. coli* degradosome, establishing a scaffold for the assembly of other degradosome subunits and performing the initial endoribonucleolytic event during substrate mRNA decay[12]. RNase E preferentially cleaves 5' monophosphorylated transcripts, thus the rate of mRNA decay is accelerated by the enzyme RppH, which converts the 5' triphosphate group to 5' monophosphate[13]. Resulting cleavage products are subsequently digested in a 3'→5' fashion by the concerted activities of the degradosome-associated exoribonuclease PNPase and RhlB RNA helicase or by degradosome-independent 3'→5' exoribonucleases, such as RNase II and RNase R[12, 14–16]. Other endoribonucleases also contribute to mRNA degradation, including RNase G, RNase I, and RNase III[17–19]. Most of these RNases cannot degrade to the single nucleotide, resulting in short RNA fragments that are further broken down by the enzyme Oligoribonuclease (Orn; 3'→5' exoribonuclease)[20]. Additionally, the endoribonuclease RNase P is known to cleave mRNA transcripts that contain riboswitches and can cleave near stem-loop structures within *E. coli* mRNAs[21–23]. Resulting cleavage products contain a 5' loop structure that acts to stabilize select transcripts[23]. RNase E, Orn, and RNase P are essential enzymes in the Gram-negative model organism *E. coli*, thus they may be good antibiotic drug discovery targets[24–26].

Researchers have long speculated that mRNA degradation within Gram-positive bacteria is also mediated by an RNA degradosome. However, the absence of an RNase E ortholog has thwarted efforts to identify this complex. Only recently, studies have revealed that *Bacillus subtilis* and *Staphylococcus aureus* are indeed capable of forming degradosome-like complexes consisting of at least eight subunits, including RNase J1, RNase J2, RNase Y (also known as CvfA and YmdA), enolase, RNA helicase (CshA), PNPase, phosphofructokinase (Pfk), and the protein component of the ribonucleoprotein complex RNase P, RnpA (Figure 1B)[27, 28]. Current studies have begun to unravel the mechanism(s) by which components of the Gram-positive degradosome subunits contribute to mRNA decay. Those studies have predominantly focused on understanding RNA degradation in the

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Gram-positive model organism, *B. subtilis*, where RNase J1, which exhibits endo- and 5'→3' exoribonuclease activities, is likely to initiate degradation. Internal cleavage is thought to be initiated by RNase J1 and resulting fragments are subsequently exonucleolytically digested by RNase J1 in the 5'→3' direction in concert with PNPase and CshA in the 3'→5' direction[27–29]. Like RNase E, RNase J1 preferentially cleaves 5' monophosphorylated mRNA, and an RppH equivalent (bsRpph or YtkD) has been identified in *B. subtilis*[30]. Another essential component of the *B. subtilis* degradosome-like complex is RNase Y, an endoribonuclease that contributes to bulk RNA degradation and has also been hypothesized to be the functional equivalent to the *E. coli* RNase E[31, 32]. *B. subtilis* transcripts are also degraded by a combination of other endoribonucleases, such as RNase J2, and the degradosome-independent RNase III[33]. Additionally, RNase P which is a ribonucleoprotein complex composed of an RNA subunit (RnpB) and a protein subunit (RnpA) affects the mRNA turnover properties of specific *B. subtilis* transcripts[21, 34, 35]. Studies in *S. aureus* have revealed that the protein component of RNase P, RnpA, affects bulk cellular mRNA turnover, as RnpA-depleted cells show increased mRNA stability, suggesting that RnpA acts to globally destabilize transcripts, albeit through an unknown mechanism[36]. RnpA is an essential member of the degradosome-like complex in both *B. subtilis* and *S. aureus*[28].

When comparing the Gram-positive *B. subtilis* and *S. aureus* mRNA degradosomes, the components are conserved, however the interactions between their subunits vary, and they have different physiological characteristics. For instance, *B. subtilis* RNase Y is an essential enzyme, but it is not required for *S. aureus* viability; conversely, RNase J2 is an essential *S. aureus* gene but allelic deletions in *B. subtilis* are not lethal[37–39]. Thus, while the overarching mechanisms by which these two Gram-positive bacteria degrade RNA molecules may be conserved, the subunits' behaviors and properties are likely to differ. In that regard, given that the latter is a life-threatening human pathogen, the essential components of the *S. aureus* degradosome may serve as the more practical and effective targets for antibiotic development. *S. aureus* RNase J1, RNase J2, and RnpA, are essential members of the organism's RNA degradation apparatus and may represent antibiotic targets.

Eukaryotic cells are highly specialized and compartmentalized, and as such, individual steps in the mRNA degradation pathway occur in distinct locations within the cell and are carried out by correspondingly unique combinations of RNases[9, 10]. Human mRNA is synthesized, 5' m^7G capped, and 3' polyadenylated within the nucleus. Degradation of the mRNA molecule can occur within the nucleus or at any point during or after transport to the cytoplasm by endonucleolytic cleavage, 5' decapping (decapping enzymes Dcp1/Dcp2), and/or removal of the 3' poly(A) tail by a variety of unique deadenylases (PAN2–PAN3, CCR4–NOT and poly(A)-specific ribonuclease (PARN)), each of which have varying roles in mRNA deadenylation among eukaryotes[10]. These events in-turn create substrates that are susceptible to 5'→3' exoribonucleases, such as XRN2 (nucleus) and XRN1 (cytoplasm), or decay in the 3'→5' direction via the exosome (Figure 1C)[10]. The core of the exosome contains two distinct structures formed by nine subunits and is thought to be essential within humans[40, 41]. The RNA-binding cap structure (Rrp4, Rrp40, and Csl4) recognizes the mRNA substrate and passes it through the hexameric ring composed of PM-Scl75 (Rrp45), Rrp41, Rrp42, Mtr3, OIP2 (Rrp43), and Rrp46[40, 42]. Although the ring structure possesses conserved exoribonuclease domains (similar to bacterial PNPase and RNase PH), it has lost the ability to directly cleave RNA[9]. Instead, the exosome coordinates the assembly of 3'→5' exoribonucleases PM-Scl100 (Rrp6) and DIS3 in the nucleus, or the 3'→5' exo- and endo-ribonuclease DIS3L in the cytoplasm, and as a unit with these RNases, degrades mRNA[41, 43–46]. Although the exosome accounts for the majority of mRNA turnover, internal cleavage of the transcript can also occur *via* endoribonucleases (SMG6), ribozymes, and the RNA interference pathway, each of which produces substrates that are susceptible to 5'→3' and 3'→5' exoribonucleases[6].

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Exploiting Essential mRNA Turnover Machinery in Bacteria

As previously mentioned, ideal antibiotics exhibit broad-spectrum antimicrobial activity against an expansive repertoire of bacterial pathogens and obviously must not be toxic to the host. In that regard, even a superficial understanding of the bacterial and host mRNA turnover pathways illustrates that many of the essential RNases involved in bacterial mRNA decay act *via* endonucleolytic cleavage, while eukaryotic decay occurs predominantly in an exonucleolytic fashion. Thus, development of agents that inhibit essential bacterial endonucleases would prevent bacterial proliferation and would be less likely to affect human mRNA turnover processes. Accordingly, Table 1 provides a comparison of the known RNases for each of the ESKAPE bacterial pathogens. Separated by Gram-stain categorization, the table compares the amino acid conservation of each RNase across pathogens (percent identity listed in parentheses), its essentiality (if known), as well as the percent predicted amino acid identity of each ribonuclease to orthologous human enzymes. A survey of these data brings to light several observations. First, there is no RNase antibiotic-development target that is essential, highly conserved across each of the ESKAPE pathogens, and also lacks similarity to human enzymes. Second, subdividing the ESKAPE pathogens based on very granular evolutionary boundaries, such as cell wall composition (Gram-staining), provides several putative RNase therapeutic targets that are essential, have low similarity to human proteins, and are well conserved across Gram-negative (Table 1A) or Gram-positive (Table 1B) organisms. Thus, one could ostensibly develop antimicrobials targeting these RNases; such agents may not be broad spectrum in the strictest sense, rather they would likely be efficacious across pathogenic species belonging to a given Gram-stain-defined boundary. There may be advantages to this approach, as the concept of targeting a subset of bacteria has been predicted to more beneficial than “broad-spectrum” antibiotics. For instance, narrow-spectrum agents may spare the host’s normal bacterial flora and reduce selective pressure, thereby minimizing the development of resistance[4]. In that regard, antimicrobial agents that target essential RNases may provide a perfect blend of broadly exhibiting efficacy against a Gram-stain-specific set of bacterial pathogens and avoiding the side effects of truly “broad spectrum” agents. Table 1 indicates that three essential RNases are conserved across ESKAPE pathogens belonging to a given Gram-stain designation, each of which also exhibits limited or no sequence and/or functional conservation to members of the human RNA turnover machinery. These RNases may represent excellent antibiotic targets and include the Gram-negative RNase E, the Gram-positive RNase J1, and the protein component of RNase P, RnpA, found in both Gram types.

As stated above, RNase E is thought to play a key role in mediating Gram-negative bacterial mRNA degradation. As the central component of the degradosome, it is essential for mRNA turnover, yet it is also required for rRNA and tRNA processing[12, 47, 48]. The enzyme is also well conserved across Gram-negative bacteria (Table 1A). Furthermore, when comparing *E. coli* RNase E to the human genome, there is no significant amino acid homology to human proteins. Thus a small molecule inhibitor of RNase E would presumably exhibit antimicrobial activity against a repertoire of Gram-negative bacterial pathogens with no predicted human toxicity.

Two Gram-positive RNases with considerable potential as antimicrobial development targets are RNase J1 and the protein component of RNase P (RnpA), both of which are essential components of the *S. aureus* degradosome-like complex (Table 1B). RNase J1 is hypothesized to be the functional analog to the *E. coli* RNase E[37], which in addition to its endonucleolytic activity, is also a 5’→3’ exoribonuclease able to degrade down to single nucleotides[49, 50]. RNase J1 is similar to RNase J2 in sequence and activity, however the essentiality of RNase J2 varies among bacterial species, suggesting that RNase J1 is the better target for antimicrobial development. RNase P is a ubiquitous ribonucleoprotein

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whose composition differs between host and pathogen[35]. Bacterial RNase P is composed of one protein (RnpA) and one RNA subunit (RnpB), whereas human nuclear RNase P contains an RNA component and up to ten different protein subunits (Rpp14, Rpp20, Rpp21, Rpp25, Rpp29, Rpp30, Rpp38, Rpp40, Pop1, and Pop5) that do not share significant amino acid similarity to bacterial RnpA[51]. In bacteria, both RnpB and RnpA are essential and have historically been considered to work in concert to aid in tRNA maturation. As elaborated below, RnpA has also been shown to contribute to *S. aureus* cellular mRNA turnover, and small molecule inhibitors of this process have been shown to have considerable therapeutic potential as antimicrobials[36]. Due to sequence divergence, it is unlikely that those small molecules will exhibit efficacy against Gram-negative bacteria, however, RnpA is also essential in many Gram-negatives of considerable healthcare concern and could be considered a target for antibiotic development in those species as well (Table 1A).

Target-based Screening for RNase Inhibitors

Once an appropriate enzyme has been selected for target-based antimicrobial drug development, the protein is typically purified and an *in vitro* functional assay is developed to accurately measure the protein's activity. This assay must be sensitive enough to detect a partial loss of enzyme activity, yet robust enough to be repeated thousands of times in the presence of individual members of chemical libraries. In addition, the assay should also be amenable to miniaturization and ideally be relatively simple in format so that it can be automated. Using such assays, literally thousands of compounds can rapidly be screened for their ability to limit the protein's activity in a high-throughput manner. Resulting inhibitory agents would represent a starting point for antimicrobial development.

One of biggest challenges in designing a high-throughput assay is to ensure that the functional assay best recapitulates the protein's essential cellular function. For example, a high-throughput screening campaign targeting "RNA elongation" failed to identify inhibitors of *S. aureus* or *E. coli* RNase activity, albeit the functional assay was not disclosed[52]. This failure was attributed to the predicted inadequacy in the structural diversity of the compound library, which consisted of 260,000–530,000 members[52]. However, a pilot screen using only the protein component of *S. aureus* RNase identified small molecule inhibitors of RnpA-mediated RNA degradation with tremendous antimicrobial therapeutic promise using a chemical library of only 29,066 compounds[36]. As proof of principle, one of those molecules, RNPA1000, exhibited antimicrobial activity against several pathogenic Gram-positive bacteria, limited human cytotoxicity, and prevented disease within an acute-lethal murine model of *S. aureus* infection[36]. Thus, in this example, it is probably not the number of screened compounds that determined success; rather it is likely contingent upon developing a functional assay that best simulates the protein's essential cellular function.

In retrospect, this theme has been reiterated over and over again in target-based screens for antimicrobials and is arguably the predominant reason genomics has failed to deliver a single novel antibiotic. The late 1990's marked the beginning of the so-called genomic era, when bacterial genomic sequences became available and were immediately mined for essential genes with predicted functions. Enzyme screening campaigns were frequently performed based on identifying inhibitors of a conveniently measurable predicted activity of a given target, such as ATP binding, without knowledge of whether the measured activity actually accounted for the enzyme's essential cellular function. Consequently, assay inhibitors did not necessarily translate to molecules that exhibited antimicrobial efficacy. Only now do we fully appreciate that a complete understanding of given target's cellular function should be a prerequisite to beginning a high-throughput screening campaign. As indicated above, much is known about bacterial cellular RNase functions, and with this

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information in hand, we argue that their essential functions can be easily assessed in a high-throughput manner.

The aforementioned *S. aureus* RnpA-mediated RNA degradation assay was performed using total or *in vitro*-transcribed bacterial RNA substrate molecules and incubating with RnpA in the presence of individual members of a small compound library. Enzyme inhibition was measured as the amount of intact RNA following the addition of RiboGreen, which fluoresces when bound to intact RNA species. A secondary gel-based RNA-degradation assay was used to distinguish *bona fide* RnpA inhibitors from high-throughput screening artifacts[36]. This technique has been used successfully for *S. aureus* RnpA, and we believe that a similar assay design has the potential to identify inhibitors of other essential endoribonucleases, such as Gram-positive RNase J1, Gram-negative RNase E, and other bacterial RnpA proteins. The lead compounds uncovered by these screens can be further investigated for their bacteriostatic or bactericidal activity, inhibitor specificity, potency, and human cytotoxicity. This information can then be used by medicinal chemists to create more effective and potent analogs for further drug development.

An Alternative Approach: Utilizing Pathogenic RNases for Drug Discovery

When considering targets for antibiotics, we have focused on those bacterial RNases that are essential for the organism's survival; however, some investigators propose that blocking virulence factor function and/or inhibiting genes required for *in vivo* survival within the host can also prevent infection. In that regard, most virulence factors and/or regulatory cascades are specific to each bacterial species, thus such inhibitory agents would demonstrate very narrow-spectrum activity, with the advantages of preserving the native flora and potentially preventing resistance mechanisms from developing[53]. Many virulence factors have been identified in bacteria, but only recently has it been appreciated that RNases control their expression. Thus, we will introduce the reader to RNases that have been shown to affect bacterial pathogenesis and discuss their likelihood as possible alternative antimicrobial targets.

The 3'→5' exoribonuclease RNase R was first characterized as *vacB* (virulence associated locus B) in *E. coli* and *Shigella flexneri*, and mutants demonstrated decreased virulence factor expression, epithelial cell invasion, and hemolytic activity[54, 55]. Further studies in *Aeromonas hydrophila* showed that RNase R mutants were significantly less virulent in a mouse model of infection[56]. Thus, the enzyme could be exploited as a target for reducing bacterial pathogenesis. However, it should be noted that the enzyme apparently does not contribute to the pathogenesis of all bacteria, as *Brucella abortus* RNase R mutant showed no difference in pathogenesis, bacterial burden, or viability in a mouse model of infection[57]. The functional variability of RNase R combined with its homology to members of the human RNA degradation machinery (Table 1), suggest that RNase R would not be an ideal target. Like RNase R, PNPase is a 3'→5' exoribonuclease that is present in both Gram-positive and –negative bacterial species. *E. coli* PNPase contributes to the regulation of outer-membrane proteins that are important for virulence, and PNPase mutants in *Yersinia* species resulted in decreased cytotoxicity in cell culture, as well as decreased virulence in a mouse model of infection[58–60]. However, inactivation of *S. enteric*a PNPase resulted in increased intracellular replication and invasion, and in *S. pyogenes*, PNPase appears to degrade virulence factor transcripts[61, 62]. Furthermore, both Gram-positive and –negative PNPase exhibit significant amino acid similarity to members of the human exosome core. Discrepancies in the contribution of PNPase to pathogenesis and its homology to human proteins limit enthusiasm for this RNase as an optimal target.

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One of the known modulators of bacterial small regulatory RNA/mRNA complexes is RNase III, which cleaves non-coding RNA-bound transcripts[63–65]. Both Gram-positive and –negative bacteria encode RNase III, however its role in virulence has been limited to studies in *S. aureus*. In an RNase III-deletion mutant of *S. aureus*, secretion of virulence factors was inhibited, the supernatants of the mutant were less toxic to human cells, and the mutant was attenuated in a peritonitis mouse model of infection[66]. Despite showing promise as an anti-pathogenesis target, limited knowledge of RNase III function across many different bacterial species, combined with its amino acid similarity to human components of the RNA degradation machinery, allow us to conclude that targeting RNase III would be problematic.

The endonuclease RNase Y is a member of the Gram-positive degradosome and is essential in *B. subtilis* but not essential for *S. aureus*[38, 39]. Nonetheless, *S. aureus* RNase Y mutant strains are highly attenuated in a silkworm model of infection and demonstrate decreased hemolysin production[67]. Likewise, the Gram-positive pathogen *Streptococcus pyogenes* RNase Y enzyme is also non-essential and is involved in virulence factor expression, adaptation to nutrient stress, and contributes to the organism's pathogenesis in several animal models of infection[68, 69]. Amino acid homology searches indicate that bacterial RNase Y does not exhibit significant sequence similarity to human proteins, and thus may be an attractive target for developing agents that prevent or reduce Gram-positive bacterial pathogenesis.

It is important to note that when considering non-essential RNases as targets for attenuating bacterial pathogenesis (or any other non-essential regulatory molecule for that matter) several additional considerations must be taken into account. First, different types of infections (i.e. abscess vs. endocarditis) are likely to involve unique subsets of virulence factors. Thus, the efficacy of agents that inhibit a particular virulence factor regulatory enzyme will likely vary widely, depending on infection type. Second, many non-essential RNases exhibit redundant activities, and as a result, high potential exists for resistance to develop, as one RNase may compensate for another RNase. Third, agents designed against virulence factors prevent pathogenesis but do not aid in eliminating the bacteria, thus these molecules would not be effective in immunocompromised patients. Fourth, implementing standard *in vitro* techniques to assess the agents' potential, such as determining the minimum inhibitory concentration (MIC), would prove difficult and may complicate inhibitor optimization.

Successful RNase Drug Development: Promise for Antibacterials

Although the focus of this review is to target RNA degradation as a means for developing antimicrobials, this concept extends beyond the bacterial realm into successful application to antiviral and anticancer drug development. For instance, one current antiviral effort is to target the human immunodeficiency virus (HIV) reverse transcriptase (RT)-mediated RNase H activity[70]. During HIV replication, the RT-associated polymerase function synthesizes a DNA copy of the viral RNA genome, and RT-associated RNase H activity subsequently degrades the parental RNA copy. As reviewed by Tramontano and Di Santo, both functions are required for viral replication, however all known RT inhibitors selectively block the polymerase function[70]. As HIV mutates at a rapid frequency, the RNase H function has been an attractive target for novel anti-retroviral development[70]. In fact, a recent screen for inhibitors of RT-associated RNase H activity was performed, and several small molecules were found to bind and inhibit RNase H activity, demonstrating little to no cytotoxicity to human cells[71].

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The dysregulation of several RNases has been implicated in the development of cancer[72]. Of these, Angiogenin (Ang) RNase activity is essential for angiogenesis[73]. Several laboratories have successfully targeted Ang using a wide variety of agents including Ang-specific monocolonal antibiodies, antisense oligonucleotides, small inhibitor peptides, as well as small molecule inhibitors that block Ang RNase activity, and all have demonstrated antitumor activity *in vitro* and *in vivo*[74–78]. These examples of developing agents that block RNases outside of the prokaryotic kingdom show that inhibition is tangible and emphasize the need for advances in developing antibacterials along the same premise.

Conclusion

At the end of the day, the number of classes of antibacterial drugs must be expanded to include new targets in order to combat highly drug-resistant bacteria, most importantly the ESKAPE pathogens. Interfering with RNA-metabolizing processes has successfully produced many antibiotics (RNA polymerase—rifampicin; ribosome—macrolides, tetracyclines, and aminoglycosides; isoleucyl-tRNA synthetase—mupirocin). Yet one aspect of bacterial RNA physiology that has not yet been exploited for antimicrobial chemotherapy is the essential process of RNA turnover *via* RNases. In that regard, we propose that small molecule inhibitors of essential bacterial RNases with little homology to human proteins will sabotage cellular global mRNA homeostasis and in turn limit prokaryotic proliferation and/or pathogenesis. As proof of principle of this concept, the successful identification and therapeutic potential of inhibitors of *S. aureus* RnpA, suggest that the approach is with merit and that additional small molecule inhibitors of bacterial ribonucleases can be identified[36]. Indeed, we have shown that RnpA inhibitors exhibit “broad spectrum” antimicrobial activity toward Gram-positive pathogens of immediate healthcare concern, efficacy in animal models of infection and limit biofilm-associated bacteria to a level that meets or exceeds currently available antibiotics[36]. Accordingly, the intent of this review is to highlight additional putative RNase antimicrobial targets and provide strategies for their exploitation, and consequently, their development as agents that will be useful for the therapeutic intervention of bacterial infections.

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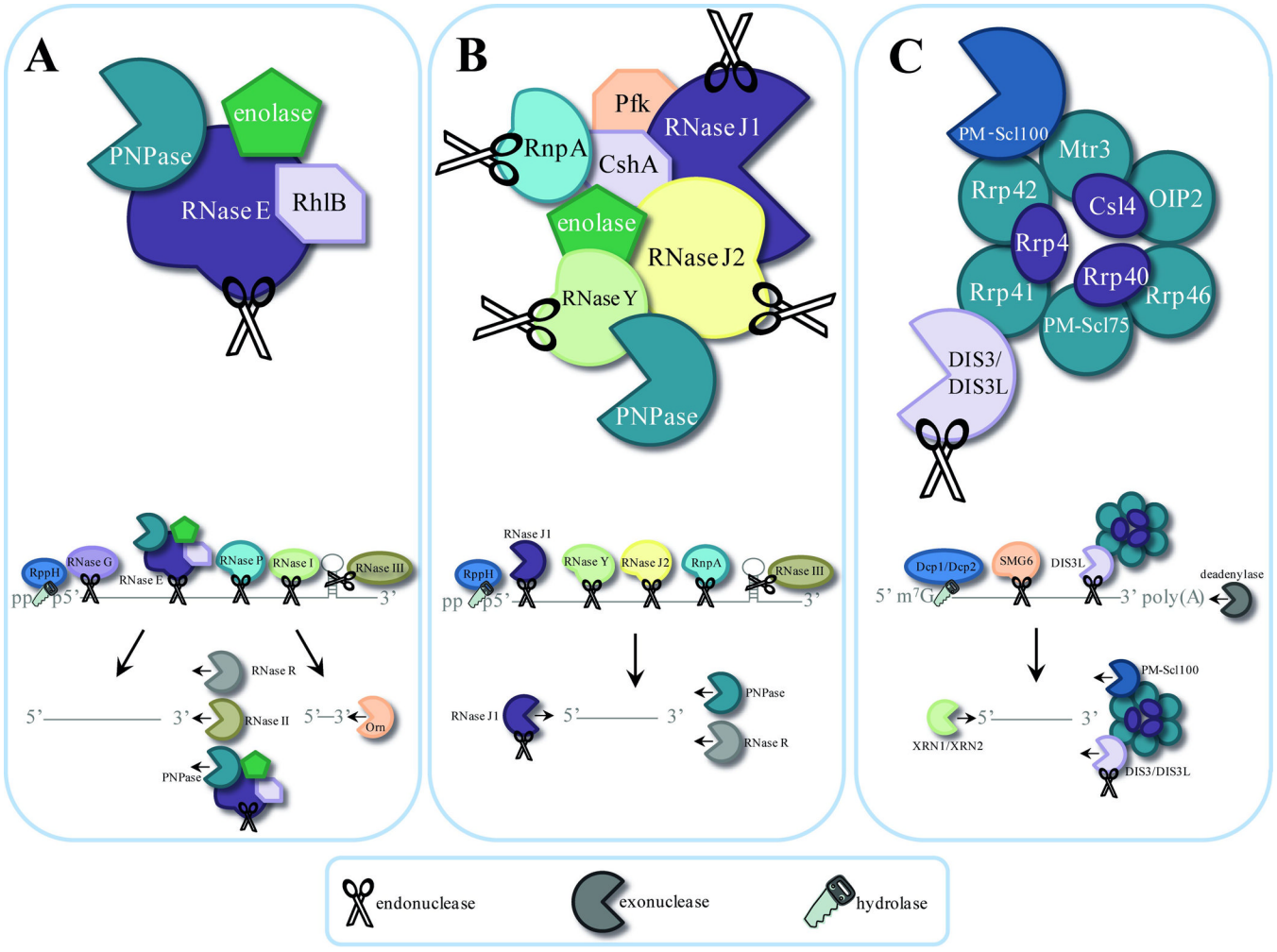

Figure 1. Mechanisms of mRNA decay

A. Model of mRNA degradosome and degradation pathways in *Escherichia coli*. The *E. coli* degradosome includes RNA helicase B (RhlB), enolase, polynucleotide phosphorylase (PNPase), and RNase E. Initiation of mRNA decay occurs with the internal cleavage by RNase E. This cleavage favors 5’ monophosphorylated transcripts, which is achieved through the action of RppH. Degradosome-independent endoribonucleases RNase G, RNase P, and RNase I cleave single-stranded RNA (ssRNA), while RNase III recognizes and cleaves double-stranded RNA secondary structures (dsRNA). Resulting cleavage products are further digested by the degradosome PNPase or by the action of RNase R and RNase II in a 3’→5’ manner into fragments that are degraded into single nucleotides by the 3’→5’ endoribonuclease Orn. **B**. Proposed model of mRNA degradosome-like complex in *Staphylococcus aureus*. The Gram-positive degradosome-like complex includes RNase J1, RNase J2, RNase Y (also known as CvfA and YmdA), enolase, RNA helicase (CshA), PNPase, phosphofructokinase (Pfk), and RnpA. In Gram-positive bacteria, internal cleavage by RNase J1 initiates mRNA degradation. RNase J1 preferentially cleaves 5’ monophosphorylated mRNA molecules that have been stripped of pyrophosphate by RppH. Other members of the degradosome, including RNase Y, RNase J2, and RnpA, also cleave transcripts endonucleolytically. Secondary dsRNA structures are recognized and cleaved by the endoribonuclease RNase III. Resulting RNA pieces are then degraded in a 3’→5’ fashion by the degradosome member PNPase and the degradosome-independent RNase R.

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Fragments are then broken down in the 5’→3’ direction by RNase J1. **C**. The human exosome contains two structures: a ring structure composed of Rrp41, Rrp42, Mtr3, OIP2, Rrp46, and PM-Scl75, and a cap structure containing Rrp4, Rrp40, and Csl4. These exosome core components associate with the 3’→5’ exoribonucleases DIS3 and PM-Scl100 in the nucleus, or the dual endo- and 3’→5’ exo-ribonuclease DIS3L in the cytoplasm. Degradation of mRNA is initiated by removal of the poly(A) tail by deadenylase activity, decapping of the 5’ end by the Dcp1/Dcp2 complex, or by the endoribonucleolytic activity of the exosome-associated cytoplasmic DIS3L. The resulting mRNA is then vulnerable to 3’→5’ degradation by the exosome-associated PM-Scl100, DIS3, or DIS3L, and 5’→3’ decay by the exoribonucleases XRN2 (nucleus) or XRN1 (cytoplasm).

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Table 1

ESKAPE pathogen ribonucleases.

A) Gram-negative[*]

Enzyme	*Escherichia coli*[24, 25, 79–90]	*Acinetobacter baumannii*[91a]	*Klebsiella pneumoniae*	*Pseudomonas aeruginosa*[91]	*Enterobacter cloacae*	Human
Oligoribonuclease	E	E (63%)	P (93%)	NE (67%)	P (96%)	REX2 (49%)
PNPase	NE	E (61%)	P (96%)	E (65%)	P (96%)	Exosome core[b] (25–33%)
RNase I	NE	P (14%)	P (70%)	-	P (79%)	RNase T2 (25%)
RNase II	NE	-	P (90%)	-	P (80%)	Dis3 (26%) Dis3L (25%)
RNase III	NE	NE (58%)	P (97%)	E (57%)	P (98%)	Drosha (30%) RNase III (30%)
RNase BN/Z	NE	P (31%)	P (69%)	NE[c] (29%)	P (77%)	tRNase Z/ELAC1 (35%)
RNase D	NE	NE (25%)	P (79%)	NE (38%)	P (82%)	PM-Scl100/Rrp6 (26%)
RNase E	E	E (61%)	P (74%)	NE (63%)	P (77%)	No known ortholog
RNase G	NE	P (54%)	P (97%)	NE (58%)	P (96%)	No known ortholog
RNase HI	NE	E[d] (49%)	P (91%)	E (62%)	P (92%)	RNase H1 (37%)
RNase HII	NE	NE (65%)	P (89%)	NE (71%)	P (91%)	RNase H2, subunit A (30%)
RNase P (RnpA)	E	P (39%)	P (97%)	NE (47%)	P (97%)	No known ortholog[e]
RNase PH	NE	E (67%)	P (96%)	NE (72%)	P (96%)	Exosome core[b] (25–28%)
RNase R	NE	NE (42%)	P (93%)	NE (53%)	P (93%)	Dis3 (29%) Dis3L (26%)
RNase T	NE	NE (54%)	P (86%)	E (63%)	P (89%)	No known ortholog

B) Gram-positive[†]

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A) Gram-negative*

Enzyme	*Escherichia coli*[24, 25, 79–90]	*Acinetobacter baumannii*[91a]	*Klebsiella pneumoniae*	*Pseudomonas aeruginosa*[91]	*Enterobacter cloacae*	Human
Enzyme	*Staphylococcus aureus*[38, 69]	*Enterococcus faecium*[92]				Human
PNPase	NE	P (63%)				Exosome core[b] (25–33%)
RNase III	NE	P (53%)				Drosha (36%) RNase III (31%)
RNase BN/Z	NE	P (53%)				tRNase Z/ELAC1 (38%)
RNase HI	NE	P (35%)				RNase H1 (35%)
RNase HII	NE	P (48%)				RNase H2, subunit A (26%)
RNase HIII	NE	P (41%)				RNase H2, subunit A (22%)
RNase J1	E	P[f] (63%)				No known ortholog
RNase J2	E	NE[f] (35%)				No known ortholog
RNase M5	NE	P (51%)				No known ortholog
RNase P (RnpA)	E	P (47%)				No known ortholog[e]
RNase R	E	P (48%)				Dis3 (31%) Dis3L (26%)
RNase Y	NE	P (63%)				No known ortholog

*Protein percent identity calculated in comparison to *E. coli* RNase

†Protein percent identity calculated in comparison to *S. aureus* RNase

E = essential
NE = non-essential
P = present, essentiality unknown

[a]Deletion studies performed in closely related *Acinetobacter baylyi*

[b]Exosome core components Rrp41, Rrp42, Mtr3, OIP2/Rrp43, Rrp46, and PM-Scl75/Rrp45

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[c] Putative metal-dependent hydrolase

[d] DnaQ

[e] Human RNase P subunits Rpp14, Rpp20, Rpp21, Rpp25, Rpp29, Rpp30, Rpp38, Rpp40, Pop1, and Pop5 have no significant similarity to *E. coli* or *S. aureus* RnpA proteins

[f] Metallo-beta-lactamase superfamily protein