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Bacterial Y RNAs: Gates, Tethers and tRNA Mimics

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Abstract

Y RNAs are noncoding RNAs (ncRNAs) that are present in most animal cells and also in many bacteria. These RNAs were discovered because they are bound by the Ro60 protein, a major target of autoantibodies in patients with some systemic autoimmune rheumatic diseases. Studies of Ro60 and Y RNAs in *Deinococcus radiodurans*, the first sequenced bacterium with a Ro60 ortholog, revealed that they function with 3' to 5' exoribonucleases to alter the composition of RNA populations during some forms of environmental stress. In the best-characterized example, Y RNA tethers the Ro60 protein to the exoribonuclease polynucleotide phosphorylase (PNPase), allowing this exoribonuclease to degrade structured RNAs more effectively. Y RNAs can also function as gates to regulate access of other RNAs to the Ro60 central cavity. Recent studies in the enteric bacterium *Salmonella enterica* serovar Typhimurium resulted in the discovery that Y RNAs are widely present in bacteria. Remarkably, the most conserved subclass of bacterial Y RNAs contains a domain that mimics tRNA. In this review, we discuss the structure, conservation and known functions of bacterial Y RNAs as well as the certainty that more bacterial Y RNAs and additional roles for these ncRNAs remain to be uncovered.

INTRODUCTION

In contrast to most bacterial noncoding RNAs (ncRNAs) (1, 2), Y RNAs were initially characterized in human cells, and only later shown to exist in bacteria. The human RNAs were discovered because they are found complexed with the Ro 60 kDa autoantigen (Ro60), a ring-shaped protein that is a clinically important target of autoantibodies in patients with two systemic autoimmune rheumatic diseases, systemic lupus erythematosus and Sjögren's syndrome (3, 4). Y RNAs and their Ro60 protein partner were subsequently shown to be present in all examined animal cells as well as in a subset of bacteria (5–13). The number of distinct Y RNAs varies between species, with most characterized organisms having between two and four (4, 5, 8, 12, 13). Although all experimentally verified Y RNAs are between 69 and 150 nt, homology searches predict that some bacterial Y RNAs may exceed 200 nt (13).

Like many ncRNAs, Y RNAs are modular. All Y RNAs contain a long stem that is formed by base pairing the 5' and 3' ends (Figure 1). Within this stem is the Ro60 binding site (14–16). Binding by Ro60 stabilizes Y RNAs, as Y RNA levels are dramatically reduced or undetectable in animal cells and bacteria lacking Ro60 (11, 13, 17–20). All characterized Y

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DISCOVERY OF Y RNAS IN *D. RADIODURANS*

The first hint that Y RNAs might be present in bacteria came from the finding that the *D. radiodurans* genome encoded a potential Ro60 ortholog (36). *D. radiodurans*, a member of the *Deinococcus-Thermus* phylum, is best known for its extreme resistance to stresses such as ionizing radiation, desiccation, ultraviolet (UV) irradiation and oxidative stress (37, 38). Studies of the Ro60 ortholog, named Rsr (Ro sixty-related) revealed that it contributed to *D. radiodurans* survival following UV irradiation (11). This role is conserved, as mouse cells lacking Ro60 are also more sensitive to UV irradiation (18, 19).

Remarkably, both Rsr and four previously unknown ncRNAs (called a, b, c, and d) encoded upstream of Rsr were found to be upregulated after UV irradiation (11). Immunoprecipitations with anti-Rsr antibodies, followed by cDNA sequencing, revealed that Rsr bound these ncRNAs, and that the most enriched ncRNA in the immunoprecipitates (RNA c) could be folded to resemble metazoan Y RNAs (Figure 1B). Specifically, this ncRNA, now called Yrn1 (Y RNA 1) and a second ncRNA, Yrn2 (Y RNA 2) that was discovered subsequently because it crosslinked to Rsr *in vivo* (13), fold to form a long stem that contains sequences known to be important for binding of vertebrate Ro60 proteins to Y RNAs (11, 13) (Figures 1B and 1C). Yrn2 is encoded upstream of Yrn1 and is synthesized as a polycistronic precursor with Yrn1 and a tRNA (13). Similar to animal cell Y RNAs, both Yrn1 and Yrn2 RNAs are unstable in *D. radiodurans* lacking Rsr (11, 13). As the remaining three *D. radiodurans* ncRNAs (a, b and d) do not appear to form this stem, they have not been designated as Y RNAs. However, these ncRNAs could contribute to the recovery of *D. radiodurans* following UV irradiation.

As more microbial genome sequences were completed, it became clear that Ro60 orthologs and by inference, Y RNAs, are present in numerous bacteria. Approximately 5% of sequenced bacterial genomes contain likely Ro60 orthologs (13, 39). Bacteria containing Ro60 are present in the majority of phyla (13, 39). However, while Ro60 orthologs are easily recognized as 400 to 600 amino acid proteins containing a TROVE domain adjacent to a von Willebrand Factor A domain (25, 26), bioinformatic identification of Y RNAs lagged behind. For example, our attempts to identify more Y RNAs by searching for primary sequence or secondary structure homology revealed likely Yrn1 orthologs in *Deinococcus ficus* and *Deinococcus maricopensis*, but not in other bacteria.

IDENTIFICATION OF ADDITIONAL Y RNAS: YRLA RNAS ARE WIDESPREAD

Characterization of Y RNAs in a second bacterium, *Salmonella enterica* serovar Typhimurium (*S. Typhimurium*), resulted in a breakthrough that allowed identification of Y RNAs in numerous bacteria. Specifically, experiments in which anti-Rsr antibodies were used to immunoprecipitate RNPs from *S. Typhimurium* lysates revealed two discrete ncRNAs in the immunoprecipitates (12). As in *D. radiodurans*, these ncRNAs were encoded within 2 kb of *rsr* (in this case, immediately 3') and transcribed in the same direction (12). Similar to all characterized Y RNAs, both *S. Typhimurium* ncRNAs could fold into secondary structures in which the 5' and 3' ends basepaired to form a long stem (Figures 1D and 1E) (12). However, while the putative Ro60/Rsr binding site in metazoan and *D.*

radiodurans Y RNAs consists of highly conserved nucleotides on both the 5' and 3' strands of the stem, the conservation was less apparent in the *S. Typhimurium* ncRNAs. Because these ncRNAs appeared to differ in some respects from other Y RNAs, we named them YrlA (Y RNA-like A) and YrlB (12).

Remarkably, by performing homology searches using the *S. Typhimurium* RNAs, we identified numerous potential Y RNAs in a wide range of bacteria and also in many bacteriophages (13). Although initial BLASTN searches with YrlB only identified likely YrlB orthologs in other *Salmonella enterica*, the initial search with YrlA identified potential orthologs in the α -proteobacterium *Rhizobium etli* and the γ -proteobacterium *Pseudomonas fulva* (13). Iterative searches with each newly identified ncRNA yielded putative YrlA RNAs in other Ro60-containing bacteria and also in some mycobacteriophages that encode a Ro60 protein (13, 40). To identify additional YrlA RNAs, we used Infernal (41) to build consensus RNA secondary structure models. In these experiments, we first collected all bacterial genomes that were annotated in GenBank as containing a TROVE-domain protein, and then removed genomes in which this protein lacked a vWFA domain and/or contained other domains. By searching the remaining genomes with the Infernal models, we identified putative YrlA RNAs in more than 250 bacteria and 22 bacteriophages (13). As more genome sequences have been released, the number of Ro60-containing species with putative YrlA RNAs has continued to increase (42, 43). Moreover, in some bacteria, multiple ncRNAs that resemble YrlA are predicted (13, 42).

As in *D. radiodurans* and *S. Typhimurium*, the vast majority of the putative YrlA orthologs are encoded within 4 kb of the gene encoding Ro60 and are predicted to be transcribed from the same DNA strand (13). Importantly, experiments in which an epitope-tagged Rsr protein was used to immunoprecipitate Rsr-containing RNPs from *Mycobacterium smegmatis* revealed that the predicted YrlA RNA was present in the immunoprecipitates, as was a second ncRNA that was named YrlB (13) (Figure 1F, 1G). Additionally, anti-Ro60 antibodies from patients with systemic lupus erythematosus were shown recently to immunoprecipitate RNPs containing the putative YrlA RNA from lysates of *Propionibacterium propionicum* (43). Thus, we consider it likely that most of the predicted YrlA RNAs exist *in vivo*.

Despite the recent success in identifying Y RNAs in numerous bacteria, additional Y RNAs remain to be discovered. Since, for some bacteria, iterative homology searching failed to identify a likely YrlA (13), these Y RNAs may diverge from current models. Moreover, the finding that biochemical experiments in *D. radiodurans*, *S. Typhimurium* and *M. smegmatis* all identified a second Y RNA that was not predicted by bioinformatics searches supports the idea that more Y RNAs remain to be found (12, 13). Also, while potential Ro60 orthologs are present in some Archaea, no archaeal Y RNAs have yet been characterized. Thus, in addition to the "metazoan-like" Yrn and bacteria-specific Yrl lineages, other Y RNA lineages may exist.

CONSERVED FEATURES OF Y RNAS: THE RO60 BINDING SITE

Since Y RNAs are defined in part by their sequence-specific binding to Ro60, the nucleotides and amino acids involved in this interaction are expected to be conserved. In the crystal structure of *Xenopus laevis* Ro60 with Y3 RNA, conserved Ro60 amino acids contact the conserved bases GGUCCGA in the 5' strand of the stem (26). In *D. radiodurans* Yrn1 and Yrn2, the corresponding sequence is GGGCCGA, with only the third nucleotide differing from the metazoan motif (Figure 1B, 1C) (11, 13). In YrlA RNAs, the most conserved sequence in the stem is GNCGAAN₀₋₁G, which occurs near the 5' end (Figures 1D-1G) (13). Although a bacterial Rsr/YrlA structure has not yet been reported, it is plausible that the central CGA of this motif corresponds to the CGA at the end of the metazoan GGUCCGA motif. Consistent with the idea that contacts to this region may be particularly important for Y RNA recognition, two amino acids that contact the CG in the *X. laevis* structure, H187 and D181 (26), are the most conserved of the Ro60 residues that contact Y RNA. Ro60 proteins mutated at D181 have not been reported; however, Ro60 proteins carrying an H187 mutation (H187S) show greatly reduced Y RNA binding in vitro and fail to stabilize Y RNAs *in vivo* (26, 31, 32).

Although sequences that can base pair with the GGUCCGA motif to form a bulged helix are conserved in metazoan and *D. radiodurans* Y RNAs, these 3' sequences [UUGACC, metazoans; UUG(C/U)CC, *D. radiodurans*] are not well conserved in YrlA and YrlB RNAs. This is consistent with the finding that in the *X. laevis* Ro60/Y3 crystal structure, there are few contacts between Ro60 and these 3' sequences, suggesting they are unimportant for Ro60 recognition (26). Consistent with the idea that the ability to form the bulged helix does not contribute strongly to Ro60 recognition, Ro60 binding partially disrupts this helix, widening the RNA major groove to allow contacts with bases on the 5' strand (16, 26). The ability of the 5' and 3' sequences to base pair could be required for another function, such as the need to maintain the terminal stem required for Exportin-5 mediated nuclear export of the human Y1 RNA (44). However, it remains unclear why some bacterial Y RNAs, such as *D. radiodurans* Yrn1 and Yrn2, have retained both the conserved nucleotides and the ability to form a bulged helix.

THE SECOND Y RNA MODULE INTERACTS WITH OTHER COMPONENTS

In addition to the stem containing the Ro60 binding site, all Y RNAs contain a second module (Figure 1). A major role of this second domain is to interact with other proteins, thus allowing the Y RNA to scaffold interactions between Ro60 and additional proteins. A number of proteins interact with one or more mammalian Ro60 RNPs by binding the large internal loops that are a prominent feature of all characterized metazoan Y RNAs (20, 45). These proteins include the multifunctional RNA-binding proteins PUF60, PTBP1 and nucleolin, the zipcode-binding protein ZBP1 and the interferon-inducible protein IFIT5 (33, 46–50). Although in most cases, the way in which the tethered protein affects Ro60 RNP function is unknown, binding of ZBP1 to mammalian Y3 RNA adapts the Ro60/Y3 RNP for Crm1-mediated nuclear export (33).

For the best characterized bacterial Y RNA, *D. radiodurans* Yrn1, structure probing supports a model in which the second module contains three stem-loops (Figure 1B) (13). This portion of Yrn1 is required for the interaction with PNPase (12). PNPase is a homotrimeric ring topped by S1 and K-homology (KH) single-stranded RNA-binding domains (51). Biochemical and structural data support a model in which single-stranded portions of this second Yrn1 module bind the PNPase S1 and KH domains (12). Interestingly, one stem-loop in this module resembles the “T arm” found in all tRNAs (13) (discussed below). As this stem-loop is not required for PNPase binding (13), it may contribute to Y RNA structure or function in other ways, such as through stabilizing Y RNA tertiary interactions (52).

YRLA RNAS CONTAIN A DOMAIN THAT MIMICS TRANSFER RNAS

Remarkably, in YrlA RNAs, the second module contains striking similarities to canonical tRNAs (13). The tRNA mimicry becomes apparent when YrlA RNA is oriented as in Figure 3A. In this orientation, the middle stem-loop of YrlA RNA corresponds to the tRNA acceptor stem and most conserved nucleotides are located within stem-loops that resemble tRNA D and T arms (Figure 3A)(13). These include many nucleotides that are invariant in tRNAs and that play critical roles in stabilizing the canonical L-shape. At least seven tertiary interactions that form in tRNA can potentially form in most YrlA RNAs (Figure 3B) (13). Consistent with the hypothesis that this YrlA domain folds to resemble tRNA, YrlA RNAs from various bacteria contain compensatory changes that maintain the “Levitt basepair” (R15:Y48) that is crucial for stabilizing the tRNA L-shape (13).

In support of the hypothesis that YrlA RNAs are tRNA mimics, these ncRNAs are substrates for some tRNA processing and modification enzymes (13). *S. Typhimurium* YrlA was shown to contain several nucleotide modifications characteristic of tRNAs, such as the pseudouridine that is at position 55(Ψ55) in the TΨC loop of nearly all tRNAs and dihydrouridine in the D-loop (Figure 3A) (13). These YrlA RNA modifications are catalyzed by the same enzymes that modify the analogous tRNA sites, as they were not detected in strains containing mutations that disrupt catalysis by TruB, the enzyme that pseudouridylates Ψ55, or by DusA, the enzyme responsible for dihydrouridine formation at the same site in tRNA (Figure 3A) (13). Moreover, in *Mycobacterium smegmatis*, the YrlA RNA stem-loop corresponding to the acceptor stem can be cleaved by RNase P, the endonuclease that matures tRNA 5' ends (13), most likely because this YrlA RNA contains a seven-base pair “acceptor stem”, the optimal length for RNase P cleavage (53). Following cleavage, the fragment corresponding to the tRNA 3' end undergoes post-transcriptional addition of CA, resulting in the CCA tail that is characteristic of all tRNAs (Figure 3C) (13).

Although the T arm of *D. radiodurans* Yrn1 also contains pseudouridine at the position corresponding to Ψ55 (13), the resemblance to tRNA is less apparent (Figure 3D). A structure with some similarity to tRNA can be drawn for Yrn1; however, the stem-loop that would correspond to the “D arm” does not contain dihydrouridine and critical tertiary interactions that stabilize the tRNA L-shape are not predicted to form (Figure 3D) (13). Thus, the only identified bacterial Y RNAs that are currently predicted to mimic tRNA are members of the YrlA family.

EVOLUTIONARY CONSIDERATIONS

Why do only some bacteria contain Ro60 RNPs? We favor a model in which Rsr and Y RNAs derive from multiple episodes of lateral gene transfer. The patchy distribution of Rsr and Y RNAs, in which they are present in a small fraction of bacteria but a majority of phyla, is consistent with a lateral transfer model, as is the finding that Ro60 and YrlA RNA are present in phages isolated from diverse species, including *Bacillus megaterium*, *Caulobacter crescentus*, *Gordonia malaquae*, *M. smegmatis*, and *Streptomyces griseus* (13, 40). In support of lateral transfer, phylogenetic trees of bacterial Ro60 orthologs are markedly different from standard phylogenetic trees based on bacterial 16S rRNA sequences (Figure 4) (39).

The striking resemblance of YrlA RNAs to tRNAs makes it likely that these ncRNAs evolved from tRNAs. Since bacterial Y RNAs are encoded adjacent to their Ro60 binding partners (13), and frequently abut tRNA genes, we hypothesize that, following acquisition of a metazoan Ro60 gene, one or more bacteria adopted a tRNA-containing transcript as a binding partner. Moreover, the fact that YrlA RNAs differ from canonical tRNAs in that the “T arm” is encoded 5’ to the “D arm” suggests that the primordial YrlA RNA derived from a dimeric tRNA. In this scenario, the T arm of the first tRNA and the D arm of the second tRNA evolved to form YrlA. This model could provide an explanation for why the “acceptor stem” of YrlA RNAs varies in sequence and length, while the T and D arms resemble *bona fide* tRNA (13). If YrlA RNAs derive from tandemly repeated tRNAs, the highly variable gap sequence between the two tRNAs would become the YrlA “acceptor stem”. In this scenario, YrlA RNAs may have evolved multiple times in bacteria. Alternatively, if YrlA RNAs derive from a single ancestral dimeric tRNA, the variability in the “acceptor stem” could reflect fewer functional constraints on this stem-loop.

FUNCTIONS OF BACTERIAL Y RNAS AND THEIR RSR PARTNERS

Rsr and Y RNAs Modulate RNA Metabolism during Environmental Stress

Since bacterial Y RNAs, like their metazoan counterparts, are bound and stabilized by Rsr (11, 13, 31), the functions of Rsr and Y RNAs are entwined. In *D. radiodurans*, a major role of Rsr and Y RNAs is to assist survival following environmental stress. *D. radiodurans* lacking Rsr are less resistant to UV irradiation than wild-type strains (11) and are at a competitive disadvantage during growth in stationary phase (54). In addition, both Rsr and Y RNA are upregulated during heat stress (31), growth in stationary phase (54), and during recovery from UV radiation, ionizing radiation and dessication (11, 55). The role of Ro60 RNPs in aiding survival after stress is conserved, as mouse cells lacking Ro60 are sensitive to UV irradiation (18) and nematodes lacking Ro60 have defects in forming dauer larvae, an alternative larval stage that developing worms form upon encountering unfavorable growth conditions (56).

Genetic and biochemical analyses revealed that *D. radiodurans* Rsr and Yrn1 RNA function with exoribonucleases to alter RNA metabolism following environmental stress. In *D. radiodurans* 23S rRNA maturation is inefficient at the normal growth temperature of 30°C, as approximately 40% of the 23S rRNA contains 5’ and/or 3’ extensions (31). Maturation of

23S rRNA becomes highly efficient when these cells are shifted to 37°C, and requires Rsr and two 3' to 5' exoribonucleases, RNase II and RNase PH (31). In this case, the Y RNA-free form of Rsr carries out maturation, and Yrn1 RNA binding to Rsr inhibits 23S rRNA maturation (31).

Additionally, Rsr and the ring-shaped 3' to 5' exoribonuclease PNPase function in rRNA degradation during prolonged growth in stationary phase (54). Rsr and Yrn1 levels increase nearly 30-fold during growth in stationary phase, compared to their levels in logarithmic phase (54). PNPase levels increase three-fold, as does formation of a complex between Rsr and PNPase (54). Consistent with a role for Rsr as an adaptor that assists rRNA degradation by PNPase, rRNA decay is less complete in strains lacking Rsr or PNPase, and sedimentation of PNPase with partially degraded ribosomal subunits requires Rsr (54).

Although the role of Yrn1 in rRNA decay was not fully explored due to its degradation in *D. radiodurans* lysates, rRNA degradation was also less efficient in strains lacking Yrn1 RNA (54). In support of a role for Yrn1, this RNA exhibits genetic interactions with Rsr and PNPase. Cells lacking PNPase (*pnp*) grow slowly at all temperatures and are sensitive to cold, oxidative stress and UV irradiation (31). Although cells lacking Yrn1 (*ym1*) grow normally, strains lacking both PNPase and Yrn1 (*pnp ym1*) show enhanced sensitivity to both low temperature and oxidative stress (31). Remarkably, although *rsr* strains also grow similarly to wild-type cells, deletion of Rsr in *pnp* and *pnp ym1* strains (*rsr pnp* and *rsr pnp ym1*) largely alleviates the sensitivity to cold and oxidative stress, indicating that the growth defects of *pnp* and *pnp ym1* strains are partly due to Rsr (31). One explanation for the genetic interactions is that, in cells lacking PNPase, binding of Y RNA-free Rsr to specific RNAs prevents their degradation by other ribonucleases and inhibits growth (31).

Y RNA Tethers PNPase to Rsr to Form RYPER

To determine how Rsr and Y RNA influence PNPase function, our laboratory purified the Rsr/Y RNA/PNPase complex from *D. radiodurans* (12). Characterization of this complex revealed that it sedimented with a molecular size consistent with one Rsr ring, one Yrn1 RNA and one PNPase trimer (12). Further analysis revealed that Yrn1 functions as a scaffold, since Rsr and PNPase bind distinct sites on this RNA (12). Specifically, while Rsr binds its high affinity site on the Yrn1 stem, the S1/KH single-stranded RNA binding domains of PNPase interact with one or more loops at the other end of Yrn1 (Figure 5A) (12). This complex was named RYPER (Rsr/Y RNA/PNPase Exoribonuclease RNP) (20). Single particle electron microscopy of RYPER revealed that the Rsr and PNPase rings were bridged by a rod-shaped density that likely represents Y RNA (Figure 5B) (12). The two rings are configured such that single-stranded RNA can thread through the Rsr ring into the PNPase cavity (Figure 5B) (12). Biochemical studies revealed that RYPER degrades structured RNAs more effectively than PNPase, but is less active in degrading single-stranded RNAs, possibly because Yrn1-mediated tethering of Rsr to the PNPase S1/KH domains impedes RNA decay substrates from binding these single-stranded RNA binding domains (12). Additionally, since vertebrate Ro60 binds RNAs containing both a single-stranded tail and helices (30), the replacement of the single-stranded RNA-binding surface

of PNPase with the Rsr binding surface may alter the target specificity of PNPase. As Rsr, PNPase and YrlA RNA all sediment as part of a complex in *S. Typhimurium*, at least some aspects of RYPER may be conserved (12).

Although RYPER is the only known RNP degradation machine, it resembles the archaeal and eukaryotic RNA degradation machine known as the RNA exosome. In archaea, the exosome consists of a heterohexameric RNase PH domain-containing catalytic ring topped by an RNA-binding ring (57). As PNPase is a trimer, in which each monomer includes two RNase PH domains, RYPER also consists of an RNA-binding ring atop an RNase PH domain-containing catalytic ring, although Y RNA tethering replaces the protein-protein interactions that join the two exosome rings (12). In yeast and animal cells, the RNase PH domains of the exosome contain mutations that render them catalytically inactive, and RNA threads through both the RNA-binding ring and the inactive RNase PH-domain ring to reach an active exoribonuclease (58). As in these exosomes, where channeling of RNA through the catalytically inactive RNase PH ring contributes to unwinding an RNA duplex (59), threading of RNA through the Rsr ring may assist ATP-independent RNA unwinding (12).

RYPER also exhibits some similarities to the bacterial degradosome, an RNA degradation machine best characterized in *E. coli*. In *E. coli* and many other γ -proteobacteria, the degradosome is a stable complex consisting of PNPase, an RNA helicase, the metabolic enzyme enolase, and the scaffolding endonuclease RNase E (60). In the degradosome, the helicase assists in unwinding structured RNA, while the endonuclease activity of RNase E generates additional ends for PNPase entry (60). Although the activity of RYPER on structured RNAs may be enhanced by endonucleases and/or helicases *in vivo*, it differs from the degradosome in that the increased activity of RYPER on structured RNAs can be observed in the absence of these enzymes (12). Because RYPER has only been well-characterized in *D. radiodurans*, an organism in which a PNPase assembly resembling the degradosome has not been described, the extent to which RYPER and the degradosome functionally overlap remains unknown. Studies in γ -proteobacteria that contain both RYPER and a degradosome-like complex, such as *S. Typhimurium* (12, 61), are needed to address this question.

Y RNAs can Regulate Access to Rsr

In addition to acting as tethers, Y RNAs can function as gates to regulate access of other RNAs to the Rsr cavity. One example of this role occurs during the heat stress-induced maturation of 23S rRNA in *D. radiodurans*. As described above, maturation of 23S rRNA is inefficient when cells are grown at 30°C, but becomes efficient at 37°C through a process that requires Rsr and two exoribonucleases (31). Consistent with a role for Yrn1 as an inhibitor, 23S rRNA maturation is efficient at all temperatures in strains lacking Yrn1 (31). Maturation is also efficient at all temperatures when Rsr carrying a point mutation that abrogates Y RNA binding is overexpressed in wild-type cells (31). Together these data indicate that the Y RNA-free form of Ro60 carries out maturation and Y RNAs inhibit this process, presumably by sterically blocking access of the pre-23S rRNA 3' extensions to the Rsr central cavity.

If a bound Y RNA can prevent entrance of other RNAs to the Ro60 cavity, how is Y RNA binding regulated? For those functions, such as 23S rRNA maturation, that require Y RNA-free Rsr (31), increased synthesis of a single primary transcript encoding both Rsr and Y RNA may result in excess Rsr, compared to Y RNA, since the mRNA encoding Rsr can be translated multiple times. For those functions in which Y RNA acts as a tether, binding of a partner protein such as PNPase to the Y RNA stem-loops may remove this module from the Rsr surface. Although the only reported bacterial Rsr structure lacks Y RNA (27), structural and biochemical experiments revealed that amino acids on the outer edge of the *X. laevis* Ro60 ring contact the Ro60 binding site in the Y RNA stem (26), while the stem-loop-containing second module is predicted to contact a basic platform that overlaps the binding site for misfolded ncRNAs (Figure 2D) (26, 30). Since our studies of *D. radiodurans* RYPER demonstrated that the stem-loop-containing module interacts with PNPase (12), binding of PNPase may remove this module from the Rsr surface, allowing decay substrates to enter the Rsr cavity.

The Organization of Rsr and Y RNA in Some Genomes Suggests a Role in RNA Repair

In some bacteria, Rsr and Y RNAs are encoded within an “RNA repair” operon. This was first observed in the γ -proteobacterium *S. Typhimurium*, where Rsr, YrlA and YrlB are encoded within the σ^{54} -regulated *rtcBA* operon, which also encodes the RtcB RNA ligase and the RtcA RNA cyclase (12). In metazoans and archaea, RtcB ligates pre-tRNA halves following intron excision (62–64). The RtcB substrates in bacteria are largely uncharacterized; however, *E. coli* RtcB re-ligates a 16S rRNA 3' fragment to the rRNA body after cleavage by the MazF toxin (65). Although expression of the *rsr-yrlBA-rtcBA* operon is tightly regulated by the adjacent RtcR transcriptional activator (12, 66), transcription of YrlA and YrlB was detected during infection of human cells (67) and the operon was also reported to be expressed during exposure to the nucleic acid interstrand crosslinker mitomycin C (66).

Comparative genomics revealed that Rsr and Y RNAs are encoded adjacent to RtcB in diverse bacteria, including some proteobacteria, firmicutes, bacteroidetes, planctomycetes and verrucomicrobia (42, 68). In some bacteria, the operon resembles that of *S. Typhimurium* in that both RtcB and RtcA are encoded downstream of Rsr and Y RNAs; however, in others, *rtcA* is absent (42). Since in a subset of bacteria that lack Rsr, RtcB is encoded adjacent to archease, a protein that enhances RtcB activity (69, 70), it has been speculated that Rsr and Y RNA may similarly augment RtcB function (42). However, while the genomic linkage of Rsr, RtcB and Y RNAs is relatively frequent in γ -proteobacteria, RtcB is not encoded adjacent to Rsr and Y RNA in the majority of bacteria that encode these components.

CONCLUSIONS AND PERSPECTIVES

Although investigations of bacterial Y RNAs are in their infancy, these studies have resulted in the identification of RYPER, an RNP machine specialized for structured RNA degradation, and in the discovery of new ways in which bacterial ncRNAs function. Future high-resolution structures of RYPER and of Rsr complexed with full length Y RNA should

elucidate both the molecular details that underlie RYPER function and those features of Y RNA structure that allow these RNAs to function as both gates and tethers. Biochemical and genetic studies will be required to elucidate the role of the YrlA RNA tRNA-like domain. Moreover, while all characterized Ro60-containing bacteria contain a second Y RNA (i.e. *D. radiodurans* Yrn2, *S. Typhimurium* YrlB, *M. smegmatis* YrlB) in which the second module contains a long hairpin closed by a pyrimidine-rich internal loop (Figure 1), the functions of these other Y RNAs remain unknown. We expect that elucidation of roles for these Y RNAs will continue to reveal new functions for ncRNAs and to provide additional insights into how bacteria adapt to their ever-changing environments.

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Figure 1.

Predicted secondary structures of a human Y RNA and the experimentally identified bacterial Y RNAs. (A) Human Y3 RNA. Modules involved in binding Ro60 and effector proteins are indicated. The portion of the stem containing the Ro60 binding site can form an alternative conformer containing a conserved bulged helix (16). In the structure of *X. laevis* Y3 complexed with Ro60 (26), the bases shown in green (GGUCCGA) are sites of specific interactions with the Ro60 protein. (B, C) *D. radiodurans* Yrn1 and Yrn2. The sequences that can form the conserved helix are boxed and the conserved “metazoan motif” GGUCCGA is colored in green. An adenine nucleotide that may represent the second A in

the “bacterial motif” is colored orange. On Yrn1, regions for Rsr binding and PNPase binding are indicated. (D, E) *S. Typhimurium* YrlA and YrlB. The GNCGAAN₀₋₁G motif is in orange. (F, G) *M. smegmatis* YrlA and YrlB. Nucleotides are colored as in (D, E).

Figure 2. Structures of Ro60 and Rsr proteins. (A) A molecular surface representation of *X. laevis* Ro60 (PDB 1YVR) colored by electrostatic surface potential. (B) A molecular surface representation of *D. radiodurans* Rsr (PDB 2NVO) colored by electrostatic surface potential. For both (A) and (B), positive potentials are in blue and negative potentials are in red (–10 kT/e to 10 kT/e). (C) Structure of *X. laevis* Ro60 bound to a misfolded 5S rRNA fragment (PDB 2I91). The helix binds the basic outer surface and the single-stranded 3' end binds in the hole. (D) Structure of *X. laevis* Ro60 bound to a fragment of Y RNA stem containing the conserved sequences required for Ro60 binding (PDB 1YVP). Positions of the 5' and 3' ends are indicated. Biochemical studies support a model in which other portions of the Y RNA contact a basic platform that overlaps with the misfolded RNA binding site (dashed line) (26, 30)

Figure 3.

YrIA RNAs contain a module that resembles tRNA. (A) *S. Typhimurium* YrIA presented to resemble a canonical tRNA. Highly conserved nucleotides between YrIA orthologs are colored orange, while conserved purines and pyrimidines are in blue. Bases shown to be modified *in vivo* (11) are indicated. AS, D, T and V denote the acceptor stem, D arm, T arm and variable arm, respectively. (B) *Escherichia coli* tRNA-Ala-GCA. Nucleotides that are conserved in YrIA RNAs are in orange. All depicted tertiary interactions can potentially form in YrIA RNAs. (C) The genome-encoded sequence of *M. smegmatis* YrIA was drawn to emphasize the resemblance to tRNA. The structure of the acceptor stem after cleavage, end nibbling and post-transcriptional CA addition *in vivo* (13) is also shown (arrow). Conserved nucleotides are colored as in (A). (D) *D. radiodurans* Yrn1 presented to resemble tRNA. Nucleotides in the T arm that are conserved between Yrn1 and YrIA RNAs are colored as in (A).

Figure 4.

Phylogenetic trees of representative Rsr-containing bacterial species. (A) Phylogenetic tree based on the sequences of 16S rRNAs (71). Each phylum is represented by a distinct color. (B) Phylogenetic tree based on the sequences of Rsr proteins. Sequence alignments were performed using Clustal Omega (72) and trees were drawn with the Phylogeny Interference Package (PHYLIP) using the maximum likelihood method (73).

degrading a structured RNA substrate, in which the 3' end threads from Rsr into the PNPase cavity for degradation, is depicted in blue.

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