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The Bacterial Ro60 Protein and Its Noncoding Y RNA Regulators

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Abstract

Ro60 ribonucleoproteins (RNPs), composed of the ring-shaped Ro 60-kDa (Ro60) protein and noncoding RNAs called Y RNAs, are present in all three domains of life. Ro60 was first described as an autoantigen in patients with rheumatic disease, and Ro60 orthologs have been identified in 3% to 5% of bacterial genomes, spanning the majority of phyla. Their functions have been characterized primarily in *Deinococcus radiodurans*, the first sequenced bacterium with a recognizable ortholog. In *D. radiodurans*, the Ro60 ortholog enhances the ability of 3′-to-5′ exoribonucleases to degrade structured RNA during several forms of environmental stress. Y RNAs are regulators that inhibit or allow the interactions of Ro60 with other proteins and RNAs. Studies of Ro60 RNPs in other bacteria hint at additional functions, since the most conserved Y RNA contains a domain that is a close tRNA mimic and Ro60 RNPs are often encoded adjacent to components of RNA repair systems.

Contents

INTRODUCTION

All cells contain numerous small ribonucleoproteins (RNPs) that function in diverse processes (16). Some small RNPs, such as RNase P, which catalyzes pre–transfer RNA (tRNA) 5′ maturation (33), and the signal recognition particle that targets nascent secretory proteins to the eukaryotic endoplasmic reticulum and integral membrane proteins to the bacterial inner membrane (2), are evolutionarily ancient, since the RNPs are ubiquitous in cells and present in all three domains of life. Other small RNPs are restricted to one or two domains of life but are found in most cells within these domains. These include the eukaryotic and archaeal small nucleolar RNPs that modify ribosomal RNA (rRNA), the eukaryote-specific small nuclear RNPs that carry out pre–messenger RNA (mRNA) splicing, and the telomerase RNP that repairs the ends of most eukaryotic chromosomes (16, 78). Most bacteria contain 6S RNA, which binds RNA polymerase to regulate transcription (114), and the transfer-messenger RNA–small protein B (tmRNA-smpB) complex, which rescues stalled ribosomes (46, 74). Bacteria also contain hundreds of less-conserved small RNA (sRNA) regulators, many of which base-pair with mRNAs to regulate their translation and/or decay (41, 52). These sRNAs are often stabilized by binding chaperone proteins such as Hfq and ProQ, which also assist base-pairing of many sRNAs with their mRNA targets (50, 92).

Another class of small RNPs, called Ro60 RNPs, are less straightforward to categorize phylogenetically. These RNPs, which consist of the Ro 60-kDa (Ro60) protein (119, 123) and noncoding RNAs (ncRNAs) called Y RNAs (47, 64), are found in all three domains of life. In eukaryotes, Ro60 orthologs are found in most animal cells as well as several fungi, green algae, and at least one choanoflagellate (98). Remarkably, Ro60 orthologs are also present in 3% to 5% of sequenced bacterial genomes, representing a majority of phyla (14, 19, 43, 98). In addition to this patchy phylogenetic distribution, the lack of concordance between phylogenetic trees based on Ro60 sequences and standard species trees based on 16S rRNA sequences (99) suggests that bacterial

Ro60 genes were spread by lateral gene transfer. A model involving lateral gene transfer, rather than selective gene loss, is supported by the finding of Ro60 and Y RNA orthologs encoded by phages that infect a diversity of bacteria and by our observation that Ro60 and likely Y RNAs are encoded on some bacterial plasmids (19). Ro60 orthologs are also present in some archaea, primarily halobacteria. Although Y RNAs have thus far eluded bioinformatic identification in some bacteria and all archaea, these ncRNAs have been found complexed with Ro60 in all species in which the RNPs have been characterized biochemically.

Most of what we know about Ro60 RNPs comes from studies in vertebrate cells and in bacteria. These RNPs were discovered because they are targets of autoantibodies in patients suffering from two systemic autoimmune rheumatic diseases, systemic lupus erythematosus and Sjögren's syndrome (3, 24, 64). Studies in frog, mouse, and human cells revealed that, in addition to Y RNAs, Ro60 binds defective and misfolded ncRNAs (20, 49, 82, 95). Structural analyses revealed that Ro60 is ring shaped (103) and that the ends of misfolded ncRNAs insert through the central cavity (37). Together, these findings implicated Ro60 in ncRNA quality control. Because the binding site of Y RNAs overlaps the misfolded ncRNA binding site (37, 103), Y RNAs were proposed to regulate access of defective RNAs to Ro60. Complementary studies in the bacterium *Deinococcus radiodurans* revealed that Ro60 functions with exoribonucleases to degrade structured RNAs during stress (21, 22, 120) and that Y RNAs both regulate and participate in these functions (21, 22). Most recently, studies in *Salmonella enterica* serovar Typhimurium (*S.* Typhimurium) and *Mycobacterium smegmatis* revealed a new class of bacterial Y RNAs that contain a domain that folds to resemble tRNA (19, 113).

In this review, we focus on Ro60 RNPs in bacteria. We discuss our current understanding of bacterial Ro60 and Y RNA structure, evolution, and function, as well as the many gaps in our understanding of these fascinating RNPs.

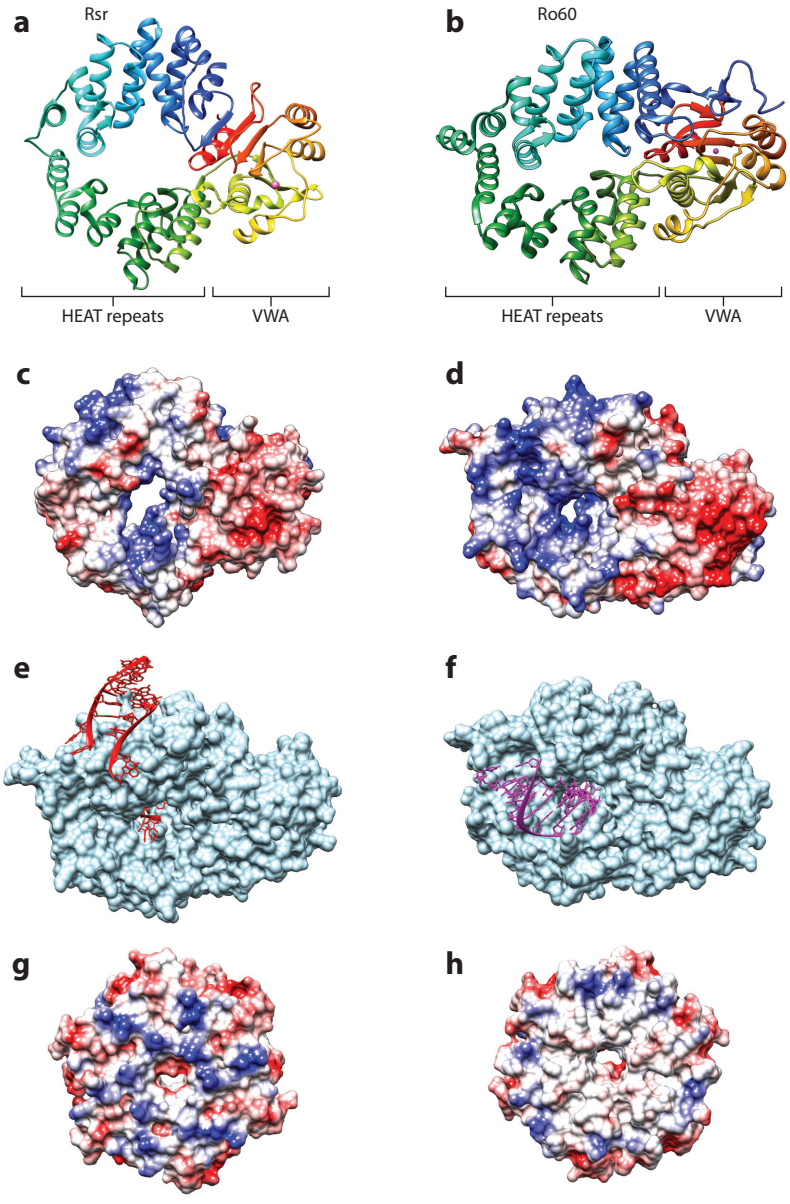
STRUCTURE OF Ro60 PROTEINS

Crystal structures have been reported of *Xenopus laevis* Ro60 and *D. radiodurans* Rsr (Ro-sixty-related) (89, 103) (**Figure 1**). In both species, the ring is formed by a series of α-helical HEAT repeats and closed by a von Willebrand factor A (VWA) domain, a protein-protein interaction motif best characterized in integrins and extracellular matrix proteins (116) (**Figure 1*a*,*b***). Within the VWA domain is a divalent cation-binding site called a metal ion–dependent adhesion site (MIDAS) that serves as a ligand-binding domain in several other proteins, including integrins, anthrax toxin receptor, and the ribosomal assembly protein Rea1 (1, 67, 91). Both the vertebrate and bacterial Ro60 proteins contain a positively charged patch on the outer rim, a cavity lined with positive charges, and a basic platform surrounding the central cavity (89, 103) (**Figure 1*c*,*d***). Although a cocrystal of Rsr with Y RNA has not been reported, a fragment of Y RNA binds to the basic patch on the *X. laevis* Ro60 outer rim, with conserved amino acids in Ro60 contacting conserved bases in the Y RNA stem (103) (**Figure 1*e***).

The central cavity and the adjacent basic platform also bind RNA. Biochemical studies revealed that Ro60 recognizes misfolded RNAs that contain both helices and a single-stranded 3′ end of at least 5 nt and that binding was not strongly sequence specific (37). In the structure of *X. laevis* Ro60 complexed with misfolded RNA (37), the single-stranded 3′ end of the misfolded RNA fragment inserts through the Ro60 cavity and a helix binds to the basic platform (37) (**Figure 1*f***). Consistent with the relative lack of sequence specificity, Ro60 primarily contacts the helix backbone (37). Although the crystal structures contain only fragments of their respective RNAs (37, 103), biochemical studies support a model in which Y RNAs and misfolded RNAs bind partially overlapping Ro60 surfaces. Since Y RNAs bind Ro60 with higher affinity than misfolded

RNAs, a bound Y RNA may sterically inhibit misfolded RNA binding (37, 103). Importantly, all surfaces shown to be important for RNA binding by *X. laevis* Ro60 are present in *D. radiodurans* Rsr (89), and many residues that contact RNA in the *X. laevis* structure are conserved in *D. radiodurans* Rsr and other bacterial orthologs (89, 103).

One difference between the *X. laevis* and *D. radiodurans* structures is in the size of the cavities (**Figure 1*a–d***). Although the *X. laevis* Ro60 cavity is 10–15 Å (103), the Rsr cavity is 18–20 Å (89). The widening of the cavity is due to the altered position of a four-helix bundle adjacent to the VWA domain (89). An intriguing possibility is that the wider Rsr cavity is due to conformational changes triggered through the VWA MIDAS. In integrins, binding of the MIDAS ligand is a



(*Caption appears on following page*)

Figure 1 (*Figure appears on preceding page*)

Structures of Ro60 proteins and their RNA ligands. Ribbon diagrams of (*a*) *Deinococcus radiodurans* Rsr (PDB 2NVO) and (*b*) *Xenopus laevis* Ro60 (PDB 1YVP). Both proteins are colored according to the rainbow with the N terminus in blue and the C terminus in red. The HEAT-repeat and VWA domains are indicated. The calcium ion in the Rsr MIDAS and the magnesium ion in the Ro60 MIDAS are indicated by pink dots. Molecular surface representation of (*c*) *D. radiodurans* Rsr and (*d*) *X. laevis* Ro60 colored according to electrostatic surface potential (−10 kT/e to 10 kT/e). Molecular surface representation of Ro60 bound to (*e*) a fragment of Y RNA containing the Ro60-binding site (PDB 1YVP) and (*f*) a fragment of misfolded 5S rRNA (PDB 2I91). In the structure with Y RNA, a single-stranded oligoribonucleotide (one of the oligonucleotides used to make the Y RNA duplex) is inserted into the Ro60 cavity. (*g*,*h*) Molecular surface representation of *Staphylococcus aureus* Hfq (PDB 1KQ2) colored according to electrostatic surface potential. The (*g*) proximal and (*h*) distal faces are shown. Abbreviations: MIDAS, metal ion–dependent adhesion site; PDB, Protein Data Bank; VWA, von Willebrand factor A.

conformational switch, triggering changes in the protein structure (67, 102). For all proteins whose ligand is known, the conserved amino acids of the MIDAS (D-x-S-x-S. . .T. . .D), together with water molecules, form most of the primary and secondary coordination spheres of the metal ion, while an Asp or Glu from the ligand completes the coordination (102). The amino acids that coordinate the divalent cation differ in the Ro60 and Rsr structures, in part because the divalent cation used for crystallization of Ro60 was magnesium (103), while for Rsr it was calcium (89). Also, although the identity of the putative ligand(s) that binds either the Ro60 or Rsr MIDAS is unknown, in Ro60 an acetate from the crystallization buffer completes the metal coordination (103), while in Rsr the coordination is completed by a Glu from a neighboring Rsr molecule in the crystal lattice that may mimic the ligand (89). Whatever the mechanism, the finding that the shape of the ring and the cavity size differ between the Ro60 and Rsr structures supports a model in which Ro60 proteins are conformationally flexible (89).

Interestingly, *D. radiodurans* Rsr was found to form oligomeric complexes upon Y RNA binding in vitro (89). These oligomers, which are greater than 700 kDa, were estimated to contain approximately 12 molecules each of Rsr and Y RNA (89). Whether similar oligomeric complexes form in cells is not known.

WHAT DEFINES A Y RNA?

In every species that has been studied, Ro60 is complexed with ncRNAs called Y RNAs. These RNAs, which were first identified in metazoans (35, 47, 54, 64, 76, 81, 83, 112, 118), are defined both by their association with Ro60 and by their ability to fold into conserved secondary structures. In animal cells, all experimentally validated Y RNAs are between 69 and 113 nt and fold into a structure consisting of two modules (**Figure 2*a***). One module consists of a long stem formed by base-pairing the 5′ and 3′ ends. Within this stem is a highly conserved sequence that is the Ro60-binding site (42, 87, 103, 119). Although this sequence can be drawn as a bulged helix in all characterized metazoan Y RNAs, with both strands of the helix nearly invariant in sequence (**Figure 2*a***), a crystal structure of Ro60 bound to a Y RNA fragment revealed that the top of the helix is frayed in the protein-bound form and that Ro60 primarily interacts with the 5′ strand (103). As Y RNA levels are reduced in both animal and bacterial cells lacking Ro60 (19, 20, 61, 117, 122), binding by Ro60 may stabilize these RNAs from degradation.

In all characterized Y RNAs, the second module consists of either a multi-branched loop or an internal loop with a single stem loop emanating from it (18, 19, 43, 83, 109, 112, 118) (**Figure 2**). Most species contain two to four distinct Y RNAs that vary primarily in this second module. Biochemical experiments support a model in which this module contacts the positively charged surface surrounding the Ro60 cavity, preventing other RNAs from accessing the cavity (37, 42, 103). Another role of this module is to tether Ro60 to other proteins (12, 21, 34, 36, 49, 100).

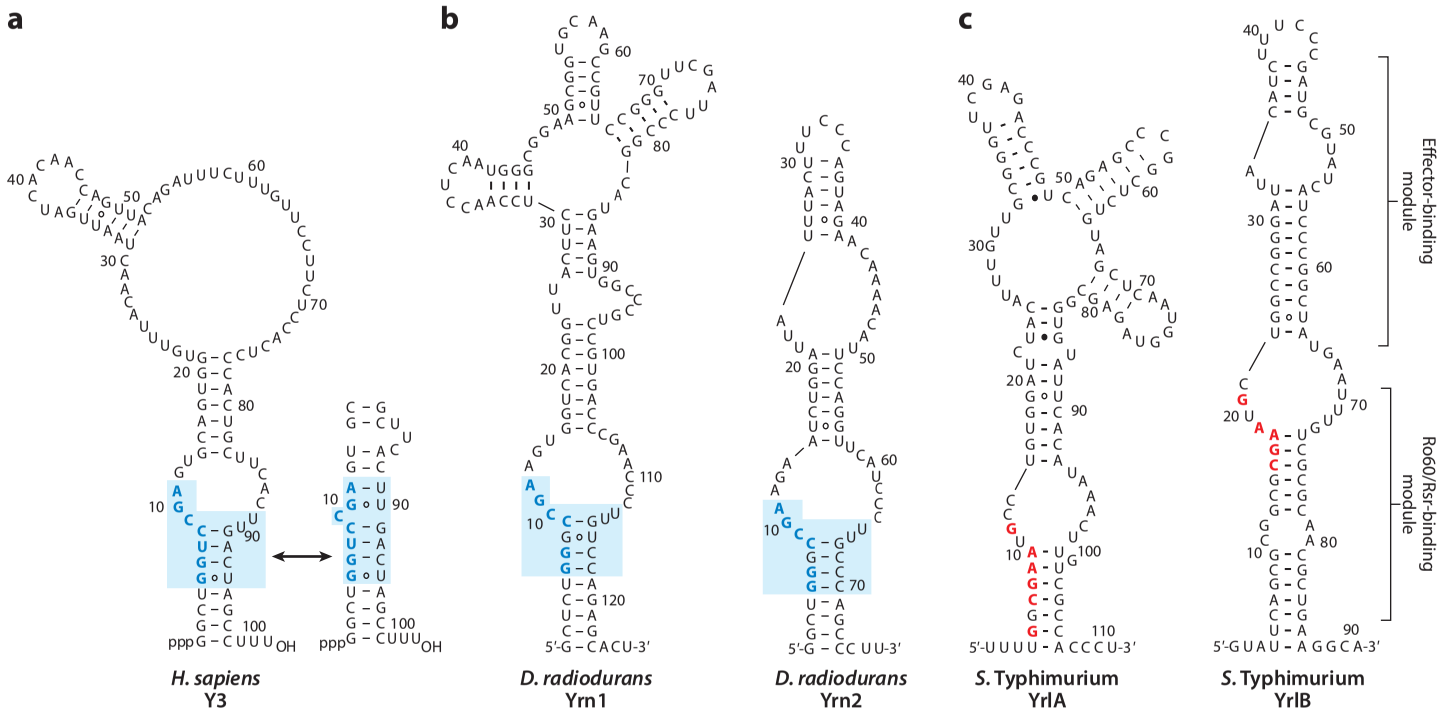


Figure 2

Secondary structures of Y RNAs. (*a*) *Homo sapiens* Y3 RNA, one of the four human Y RNAs. Y RNAs consist of two modules, one that binds Ro60 and one that binds effector proteins. Boxed sequences within the stem are critical for Ro60 binding (42) and conserved in all characterized metazoan Y RNAs. This portion of the stem can form an alternative conformer in which the conserved sequences are within a bulged helix (42). Bases that are sites of specific interactions with Ro60 in the *Xenopus laevis* Ro60/Y3 crystal structure (103) are colored blue. (*b*) *Deinococcus radiodurans* Yrn1 (*left*) and Yrn2 (*right*). The conserved sequences that resemble the metazoan Ro60-binding site are boxed and colored as in panel *a*. (*c*) *Salmonella enterica* serovar Typhimurium YrlA (*left*) and YrlB (*right*). The conserved sequence in YrlA RNAs ($GNCGAAN_{0-1}G$), which may correspond to the Rsr-binding site, is colored red.

Since several proteins associate with Ro60 by binding to a specific Y RNA, the multiple Y RNAs in most species may allow Ro60 to form complexes with a greater diversity of partners.

BACTERIAL Y RNAs

Some Bacterial Y RNAs Resemble Metazoan Y RNAs

The first bacterial Y RNA (now called Yrn1, for Y RNA 1) was discovered because it was present in anti-Rsr immunoprecipitates from *D. radiodurans* lysates. This RNA, which is encoded ~1,500 nt upstream of Rsr and transcribed in the same direction (18), is upregulated under all conditions that upregulate Rsr (18, 22, 105, 120), supporting the idea that it is part of the same operon. A second Y RNA, Yrn2, which is transcribed as a polycistronic transcript with Yrn1 and a tRNA, was identified because it cross-linked to Rsr in vivo (19). Both Yrn1 and Yrn2 resemble metazoan Y RNAs in that they contain the conserved bulged helix that is important for Ro60 binding in vertebrates (42, 87, 103, 119) (**Figure 2*b***). Similar to vertebrate Y RNAs, Rsr is important for stable accumulation of Yrn1 and Yrn2, as these RNAs are greatly decreased in strains deleted for Rsr or carrying a mutation that disrupts Y RNA binding (19, 22). Three other ncRNAs are encoded between Rsr and the two Y RNAs and appear to be cotranscribed in response to stress (18). Because these ncRNAs only weakly bind Rsr and their predicted structures differ from those of canonical Y RNAs, they are not currently designated as Y RNAs.

The Yrl Family of Y RNAs Is Widespread in Bacteria

A second class of bacterial Y RNAs was identified in the enteric gammaproteobacterium *S.* Typhimurium. These ncRNAs, which were discovered because they were present in anti-Rsr immunoprecipitates, are similar in size to other Y RNAs and can fold into secondary structures containing long stems formed by base-pairing the 5′ and 3′ ends (21) (**Figure 2*c***). These RNAs, like *D. radiodurans* Y RNAs, are encoded adjacent to Rsr (in this case, immediately downstream), are transcribed in the same direction, and appear to be part of the same operon (21). However, they lack the conserved bulged helix that encompasses the Ro60/Rsr-binding site in metazoan and *D. radiodurans* Y RNAs. Because they appeared distinct from canonical Y RNAs, these ncRNAs were named YrlA (Y RNA–like A) and YrlB (21).

Although homology searches with *D. radiodurans* Yrn1 and Yrn2 only identified potential RNAs in other deinococci, searches with *S.* Typhimurium YrlA identified likely orthologs outside *Salmonella* (19). With the use of Infernal (79) to build consensus models from the putative YrlA orthologs and search bacterial genomes, YrlA-related sequences were identified in more than 250 bacteria and 22 bacteriophages (19). Similar to the *D. radiodurans* and *S.* Typhimurium RNAs, the predicted ncRNAs were nearly all encoded within 4 kb of the Rsr ortholog in each species and transcribed in the same direction (19). Immunoprecipitations from *M. smegmatis* lysates revealed that both the predicted YrlA and a second ncRNA (named YrlB) were present in the immunoprecipitates (19). Similar experiments using human anti-Ro60 antibodies to immunoprecipitate from *Propionibacterium propionicum* lysates revealed that the putative YrlA RNA was present in the immunoprecipitates (43).

A conserved feature of all Yrl RNAs is a short sequence that resembles the 5′ strand of the metazoan Ro60-binding site. Specifically, these RNAs contain the sequence GNCGAAN$_{0–1}$G (where N is any nucleotide) in the 5′ portion of the stem formed by base-pairing the 5′ and 3′ ends (19) (**Figure 2*c***). This sequence resembles the GGUCGA that is the site of specific, high-affinity contacts between *X. laevis* Ro60 and Y RNA (103). Consistent with the hypothesis that the central CGA of the Yrl motif is analogous to the CGA of the metazoan motif, two amino acids that contact

the CG, *X. laevis* H187 and D181, are nearly invariant in Ro60/Rsr proteins (43, 103). Although the role of D181 has not been examined, mutation of either vertebrate H187 or the analogous histidine in *D. radiodurans* Rsr (H189) disrupts Y RNA binding (22, 97, 103).

The Effector-Binding Domain of YrlA RNAs Resembles tRNA

Remarkably, the effector-binding module of YrlA RNAs is a tRNA mimic. The most conserved sequences exhibit high similarity to T and D arms of canonical tRNAs, and the entire module can be folded to resemble tRNA (19) (**Figure 3*a***). These RNAs contain modifications characteristic of tRNAs and are substrates for several tRNA modification enzymes (19). A high-resolution crystal structure revealed that the effector-binding domain of *S.* Typhimurium YrlA folds into the characteristic tRNA L shape (113) (**Figure 3*c***). The structure also showed that many tertiary interactions present in tRNA are present in YrlA (113), including the elbow formed by interactions between the tRNA D and T stem loops (125), the Levitt base pair that brings together the tRNA D and variable stem loops (65), and three stacked base triplets found in tRNA (113) (**Figure 3**). However, the stem corresponding to the tRNA acceptor stem is usually a closed loop in YrlA RNAs (19), precluding aminoacylation, and the YrlA stem corresponding to the tRNA anticodon stem is connected to the Rsr-binding site (113).

The role of the YrlA effector-binding domain is currently unknown. Since the elbow in tRNAs and other tRNA-like elements can interact with the ribosome, with riboswitches, and with tRNA-binding proteins (125), this region of YrlA RNAs could be a site of RNA or protein interactions. However, the conservation within the YrlA tRNA-like domain extends beyond elements that are invariant in tRNAs. Specifically, the nucleotides that comprise the D and T stems are nearly identical in all known YrlAs, but they are not strongly conserved in tRNAs (19, 113). As no proteins have been described that recognize tRNAs through sequence-specific interactions with these stems, it is unclear why these sequences have been maintained in YrlA RNAs (113).

EVOLUTIONARY RELATIONSHIPS

Most Bacterial TROVE Domain Proteins Are Ro60 Orthologs

Ro60 proteins are easily identified because they consist of an N-terminal TROVE domain and an adjacent VWA domain containing a MIDAS and are ~400 to 600 amino acids in length (**Figure 4*a***). The name TROVE (telomerase, Ro, vault), which refers to the HEAT repeat domain, was coined because these domains of Ro60 and TEP1, which was proposed to be a telomerase subunit (45) but is now known to be a part of RNPs called vaults (57), are related in sequence (8, 88). TEP1 also resembles Ro60 in that the TROVE domain is followed by a VWA domain (8), and the TROVE/VWA portion of TEP1 can be modeled to form a ring (58, 103). TEP1 binds ncRNAs called vault RNAs, which resemble Y RNAs in size and architecture (57, 58). However, amino acids in the Ro60 TROVE domain that are required for Y RNA binding are not present in TEP1 (103), and the TEP1 VWA domain lacks a MIDAS (58) (**Figure 4*a*,*b***). TEP1 proteins also contain C-terminal WD40 repeats, which are absent in Ro60 proteins (45, 58) (**Figure 4*a***). The vast majority of bacterial TROVE domain–containing proteins are orthologs of Ro60 rather than TEP1, since these proteins show conservation of amino acids critical for Y RNA binding, contain a MIDAS within the VWA domain (**Figure 4*b***), and lack WD40 repeats.

Evolution of Y RNAs

Although the high sequence conservation of Ro60 proteins between animal cells and bacteria (43, 89, 103) makes it likely that these proteins derived from a single ancestor, bacterial Y RNAs

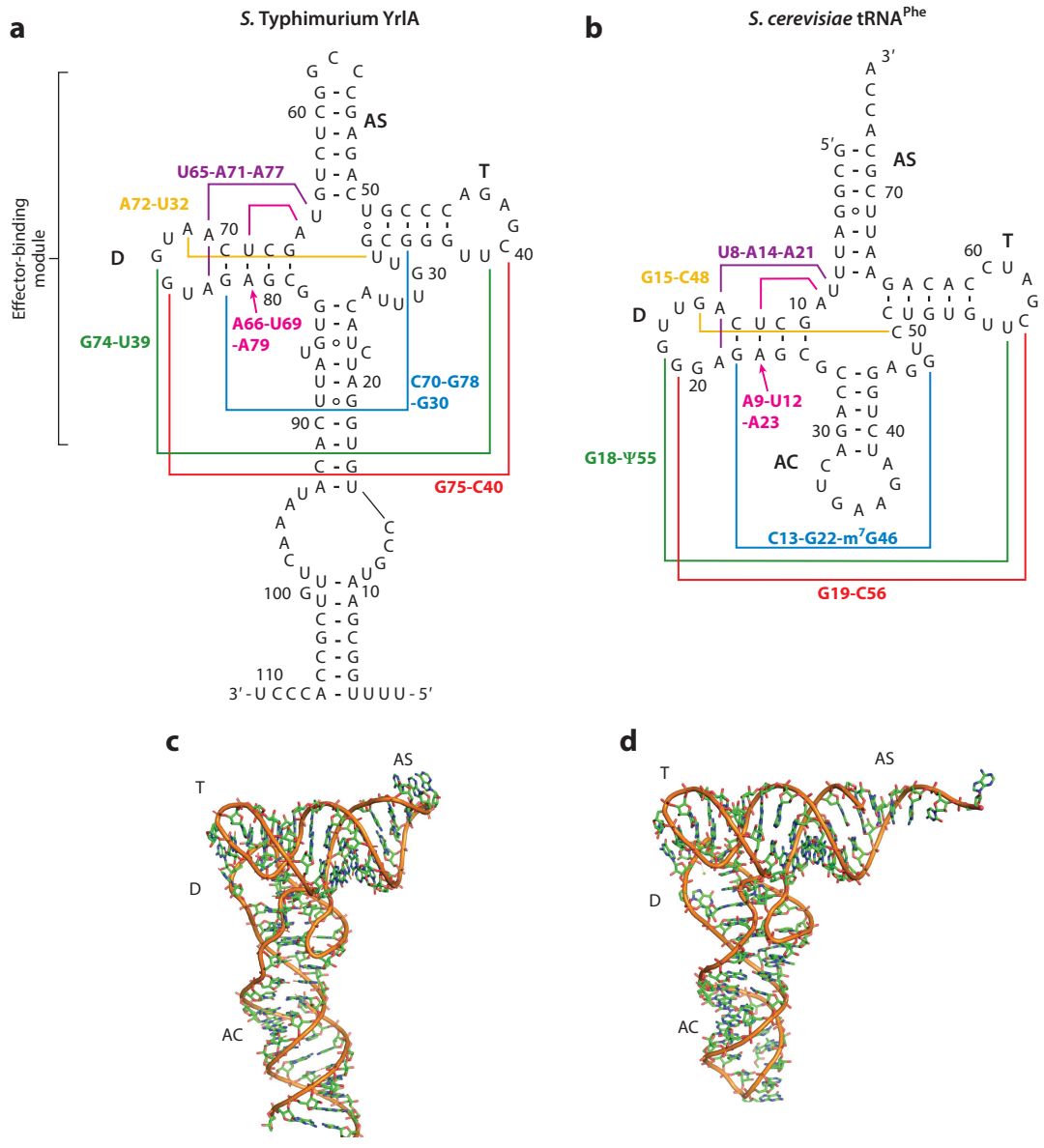


Figure 3

The effector-binding domain of YrlA RNAs resembles tRNA. (*a*) The secondary structure of *Salmonella enterica* serovar Typhimurium YrlA is oriented to resemble a canonical tRNA, (*b*) *Saccharomyces cerevisiae* $tRNA^{Phe}$. Regions corresponding to the D arm (D), T arm (T), acceptor stem (AS), and anticodon stem (AC) are indicated. Modified nucleotides are not shown in the structures. (*c*) Crystal structure of the tRNA-like domain of *S.* Typhimurium YrlA (PDB 6CU1). (*d*) Crystal structure of *S. cerevisiae* $tRNA^{Phe}$ (PDB 4TNA). Tertiary interactions that form in both structures are indicated in panels *a* and *b*. Abbreviation: PDB, Protein Data Bank.

are less related to their animal cell counterparts in primary sequence, with the exception of the motif required for Ro60 binding. Since all characterized bacterial Y RNAs are encoded adjacent to a gene for a Ro60 ortholog, often with one or more tRNA genes in the immediate vicinity (18, 19, 99), we consider it likely that these RNAs originated from tRNAs (19, 99, 113). The idea that

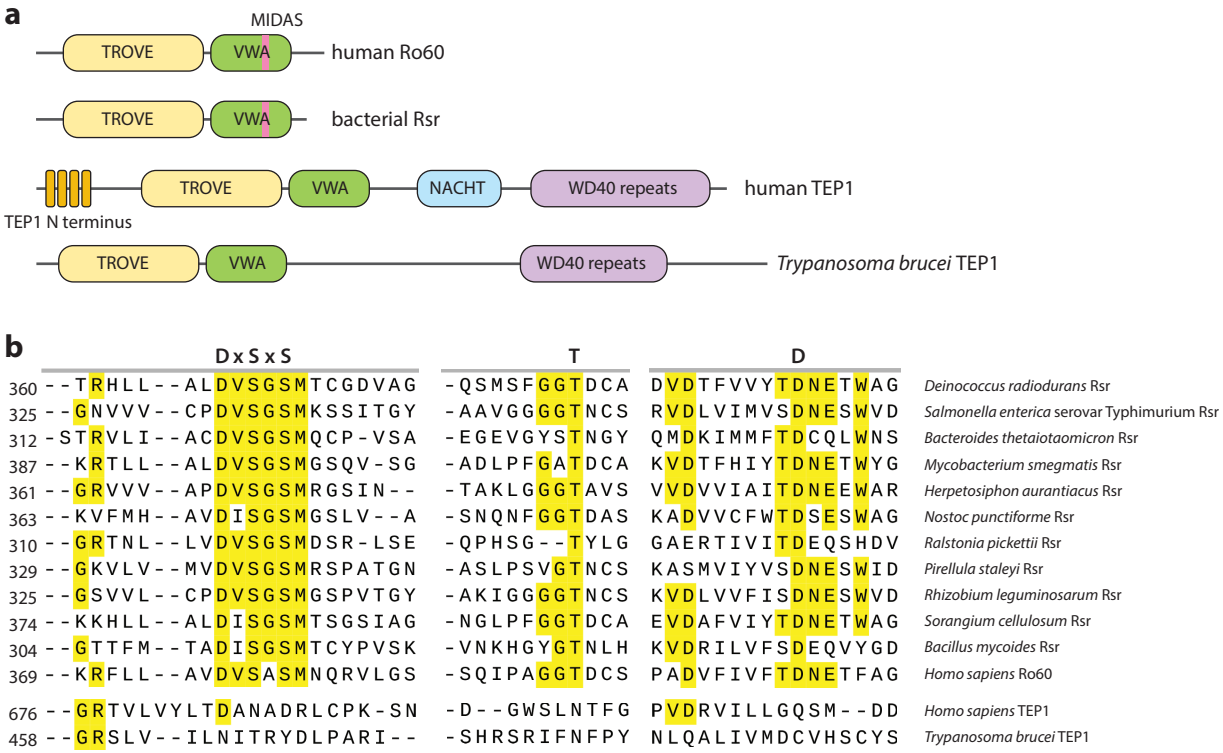


Figure 4

Bacterial TROVE domain proteins are Ro60 orthologs. (*a*) Domain organization of human and bacterial Ro60 proteins compared with that of human and *Trypanosoma brucei* TEP1. Many mammalian TEP1 proteins contain, in addition to the TROVE and VWA domains, four copies of a 29-nt sequence at their N termini, and many TEP1 proteins also contain an ATP-binding motif called a NACHT domain. (*b*) Alignment of the MIDAS motifs of human and bacterial Ro60 proteins. Amino acids highlighted yellow are conserved in >60% of sequences. The amino acids that define the MIDAS (D-x-S-x-S. . .T. . .D) are indicated at the top of the alignment. Human and *T. brucei* TEP1 proteins are also shown to demonstrate the lack of conservation with the Ro60 MIDAS. Abbreviations: MIDAS, metal ion–dependent adhesion site; TROVE, telomerase, Ro, vault; VWA, von Willebrand factor A.

bacterial Ro60 proteins adopted tRNA as a binding partner is supported by a tRNA-like T arm in *D. radiodurans* Yrn1 (19) and the presence of a tRNA-like module in YrlA RNAs (**Figure 3**). Since the order of tRNA stems is permutated in YrlA RNAs, with the T arm located 5′ to the D arm and the acceptor stem closed by a loop, these RNAs may have originated from a dimeric tRNA transcript. In this scenario, the YrlA acceptor stems, which are less conserved in length and sequence than the D and T arms, derive from sequences between the two tRNAs (99). The relative lack of conservation between YrlA acceptor stems could signify that these RNAs derive from multiple independent acquisitions of dimeric tRNAs or reflect less evolutionary pressure to maintain the sequence and/or length of these stems.

Rsr and Y RNAs Are Often Encoded Adjacent to Genes for RNA Repair Enzymes

Genes encoding proteins that function in the same pathway are often clustered in prokaryotic genomes (26). In many bacteria, including some *Proteobacteria*, *Planctomycetes*, and *Verrucomicrobia*, Rsr and Y RNAs are encoded in an RNA repair operon that also encodes RtcB, an RNA ligase that

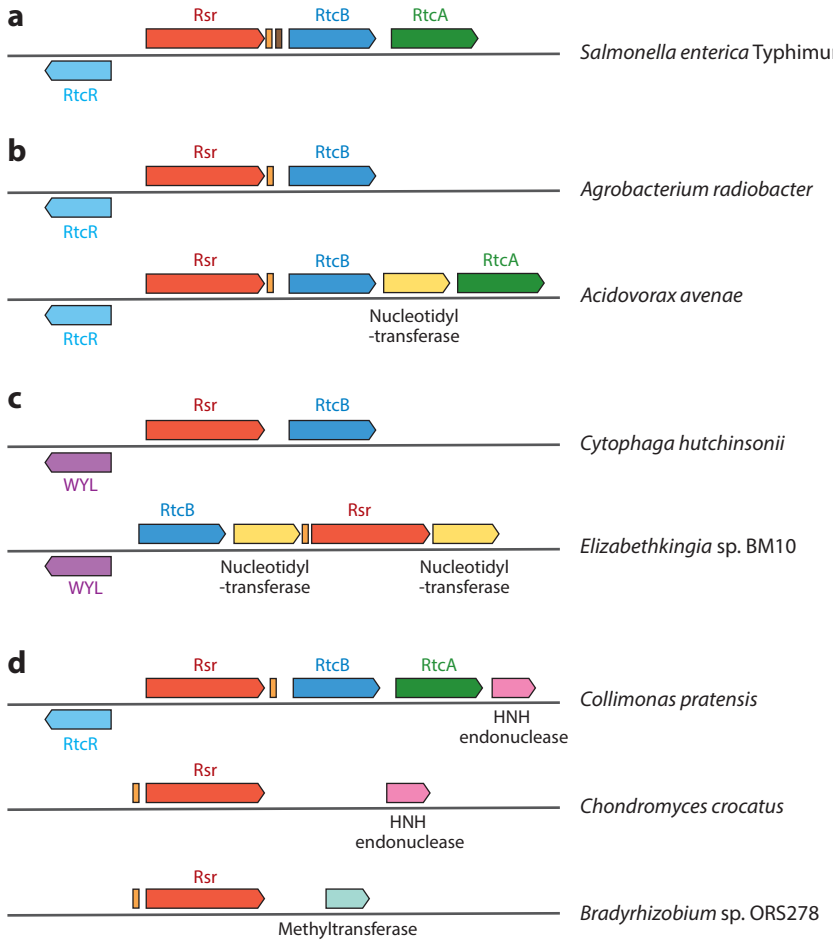


Figure 5

Gene neighborhoods of Rsr and Y RNA orthologs. (*a–d*) Examples of gene neighborhoods. Box arrows indicate the direction of transcription. Gene orthologs are indicated by color: Rsr, red; RtcB, dark blue; RtcA, green; RtcR, light blue; nucleotidyltransferase, yellow; WYL domain–containing protein, purple; HNH family endonuclease, pink; methyltransferase, cyan. In those genomes in which YrlA has been identified, this RNA is designated by an orange rectangle. *Salmonella enterica* serovar Typhimurium YrlB RNA is indicated by a brown rectangle in panel *a*. *Agrobacterium radiobacter* (*b*) and *Bradyrhizobium* sp. ORS278 (*d*) are members of the *Alphaproteobacteria*, *Acidovorax avenae* (*b*) and *Collimonas pratensis* (*d*) are members of *Betaproteobacteria*, *Cytophaga hutchinsonii* (*c*) and *Elizabethkingia* sp. BM10 (*c*) are members of *Bacteroidetes*, and *Chondromyces crocatus* (*d*) is a member of the *Deltaproteobacteria*.

joins RNAs ending in 3′ phosphate or 2′,3′ cyclic phosphate with RNAs ending in 5′OH (106) and RtcA, which converts RNAs ending with 2′ or 3′ phosphates to 2′,3′ cyclic phosphates (14, 21, 27, 38) (**Figure 5*a***). In metazoans and archaea, RtcB joins pre-tRNA halves following intron excision (32, 59, 85). Although the substrates of RtcA and RtcB in bacteria are largely unknown, RtcB is proposed to ligate specific 16S rRNA fragments produced by MazF toxin cleavage during environmental stress (108). The expression of the *rtcBA* operon is regulated by RtcR, a σ^{54}-dependent transcription activator that is usually encoded upstream of *rtcBA* (14, 21, 27, 38). Because RtcR contains a divergent CRISPR-associated Rossman fold (CARF) domain (69), which binds cyclic

oligoadenylates in some type III CRISPR-associated systems (56, 80), it is proposed that the ligand that activates RtcR is a nucleotide derivative (69). Consistent with this hypothesis, the *Escherichia coli* operon (which lacks Rsr and Y RNAs) is activated when toxins that cleave tRNAs are ectopically expressed (31), and the *S.* Typhimurium operon is activated when cells are exposed to the nucleic acid cross-linking agent mitomycin C (60).

In other bacteria, including some *Proteobacteria*, *Firmicutes*, and *Bacteroidetes*, Rsr and Y RNA are encoded adjacent to the gene for RtcB, but RtcA and/or RtcR is missing, and/or other proteins are present (**Figure 5*b***). For example, in some bacteria, RtcA is replaced by a member of the Polβ family of nucleotidyltransferases, while in others both RtcA and this nucleotidyltransferase are present (14). In some *Bacteroidetes* and *Firmicutes*, RtcR is replaced by a likely transcriptional regulator containing a WYL domain (**Figure 5*c***), which like the CARF domain is predicted to bind nucleic acid (14, 69, 77). Despite the diversity of genome organization, it is remarkable that Rsr and RtcB are found together in remotely related species across several phyla. We note, however, that in other species within the same phyla, Rsr is encoded separately from RtcB. This observation may provide additional evidence that Rsr genes have spread by lateral transfer.

Why might Rsr and RtcB be frequently coregulated? A common neighbor of RtcB in metazoans, archaea, and some bacteria is archease, a protein that enhances the activity of RtcB in tRNA ligation by allowing multiple turnover reactions (28, 86). Since in some bacteria archease is absent and Rsr and Y RNAs are encoded adjacent to RtcB, it has been speculated that Rsr and Y RNA may similarly enhance the activity of RtcB in RNA repair (14). However, in some bacteria, Rsr is encoded adjacent to components of other RNA repair systems (14) such as the Hen1 RNA 3′ methyltransferase and the RNA ligase Pnkp, which function together in RNA repair (17, 71). Thus, another possibility is that Rsr plays a more general role in RNA ligation and/or degradation of RNAs that fail to be repaired.

Rsr is also encoded adjacent to genes for other proteins with potential roles in RNA repair or decay (**Figure 5*b–d***). Since members of the Polβ family of nucleotidyltransferases add nucleotides to the 3′ ends of RNAs or DNAs (70), one possibility is that the nucleotidyltransferase that is often adjacent to Rsr and RtcB could function to repair RNA ends. Alternatively, since vertebrate Ro60 binds structured RNAs with single-stranded 3′ ends (37), this nucleotidyltransferase could function with Rsr to assist RNA decay, similar to the eukaryotic terminal nucleotidyltransferases that add short A or U tails to the 3′ ends of structured RNAs to assist degradation by 3′-to-5′ exoribonucleases (9). Members of the HNH family of endonucleases are also sometimes encoded adjacent to Rsr and RtcB genes (**Figure 5*d***). Some family members, including several Cas9 endonucleases (30, 84, 90, 104), can cleave both RNA and DNA. Since in some bacteria the methyltransferase and/or HNH endonuclease is encoded adjacent to Rsr but RtcB and other ligases are absent (**Figure 5*d***), their potential functions in RNA repair or decay could involve Rsr.

In the *Deinococcus-Thermus Meiothermus silvanus*, a member of the RNase II family of 3′-to-5′ exoribonucleases is encoded 43 bp downstream of Rsr and transcribed in the same direction (96). Since Rsr functions with 3′-to-5′ exoribonucleases to degrade structured RNA in *D. radiodurans* (21, 22), the finding that Rsr may be coregulated with RNase II hints at a similar role in *M. silvanus*. Intriguingly, this potential operon, which is encoded on an *M. silvanus* plasmid, is adjacent to a type III-B cluster of CRISPR-associated genes and encoded on the same DNA strand (96). As this effector complex carries out endonucleolytic cleavage of RNA targets (10, 44), it is tempting to speculate that Rsr and RNase II contribute to degradation of the cleaved RNAs. However, as this gene order is thus far known to be unique to *M. silvanus*, it is unlikely to be a general role of Rsr.

FUNCTIONS OF RSR AND Y RNAs

Rsr and Y RNAs Are Part of the Cellular Response to Stress

In both bacteria and animal cells, Ro60 and Y RNAs contribute to survival during environmental stress (18, 20, 120, 122). *D. radiodurans* cells lacking Rsr are more sensitive to UV irradiation than wild-type cells (18) and exhibit decreased survival during prolonged growth in stationary phase (120). Consistent with a role in enhancing survival, Rsr increases 4-fold following UV irradiation and 30-fold during growth in stationary phase, with concomitant increases in Y RNAs (18, 120). Upregulation of both Rsr and Y RNA also occurs during heat stress (22) and recovery from ionizing radiation and desiccation (105), although a role in assisting survival during these stresses has not been reported. Consistent with a role in stress, the *S.* Typhimurium operon encoding Rsr and Y RNAs is upregulated following oxidative stress, nitrogen limitation, and treatment with compounds that cross-link nucleic acids (60).

Rsr and Y RNA Function with Exoribonucleases

Studies of *D. radiodurans* Rsr RNPs during heat stress and growth in stationary phase revealed that Rsr and Y RNA function with exoribonucleases to alter RNA populations (**Figure 6**). During normal growth at 30°C, 23S rRNA maturation is inefficient, since approximately 40% of this rRNA contains 5′ and/or 3′ extensions (22). At the heat stress temperature of 37°C, the RNA is matured through a pathway that requires Ro60 and the 3′-to-5′ exoribonucleases RNase PH and RNase II (22) (**Figure 6*a***). Because 23S rRNA maturation is efficient at both temperatures in strains that either lack Yrn1 or contain a mutant Ro60 that cannot bind Yrn1 (22), pre–23S rRNA maturation likely involves the Y RNA–free form of Ro60, with Yrn1 functioning as an inhibitor (22) (**Figure 6*a*,*b***).

During stationary phase, *D. radiodurans* Rsr functions with the 3′-to-5′ exoribonuclease polynucleotide phosphorylase (PNPase) to degrade rRNA. Although massive rRNA degradation occurs within three days in stationary phase, most likely as a response to nutrient exhaustion, degradation is less complete in strains lacking Rsr, Yrn1, or PNPase (120). Because the sedimentation of PNPase with partially degraded ribosomal subunits required Rsr, Rsr was proposed to function as an adaptor to increase the interaction of PNPase with rRNA (120). Since Yrn1 was partly degraded in these experiments, its role was not assessed (120).

RYPER, an RNA Degradation Machine

Characterization of the *D. radiodurans* Rsr/PNPase complex revealed that it is scaffolded by Yrn1 (21). In this complex, called RYPER (Rsr/Y RNA/PNPase exoribonuclease RNP) (21, 117), Rsr binds the Yrn1 stem, while the KH/S1 single-stranded RNA-binding domains of PNPase bind loop(s) at the other end of Yrn1 (21) (**Figure 6*c*,*d***). PNPase is ring shaped, and single-particle negative-stain electron microscopy supported a model in which the 3′ ends of RNA substrates thread through the Rsr ring into the PNPase cavity (21) (**Figure 6*c*,*d***). Biochemical assays revealed that RYPER is more efficient in degrading structured RNA than PNPase, possibly because passage through the Rsr ring assists ATP-independent RNA unwinding (21). Yrn1-mediated tethering of Rsr to the KH/S1 domains of PNPase may also prevent single-stranded RNA substrates from binding these domains. If Rsr, like Ro60, binds structured RNAs with single-stranded 3′ ends (37), replacement of the RNA-binding surface of PNPase with that of Rsr would further specialize PNPase for structured RNA decay (**Figure 6*d***).

The discovery of RYPER revealed a new way in which exoribonucleases such as PNPase can be adapted to degrade RNAs that would otherwise be poor substrates. PNPase requires a 3′

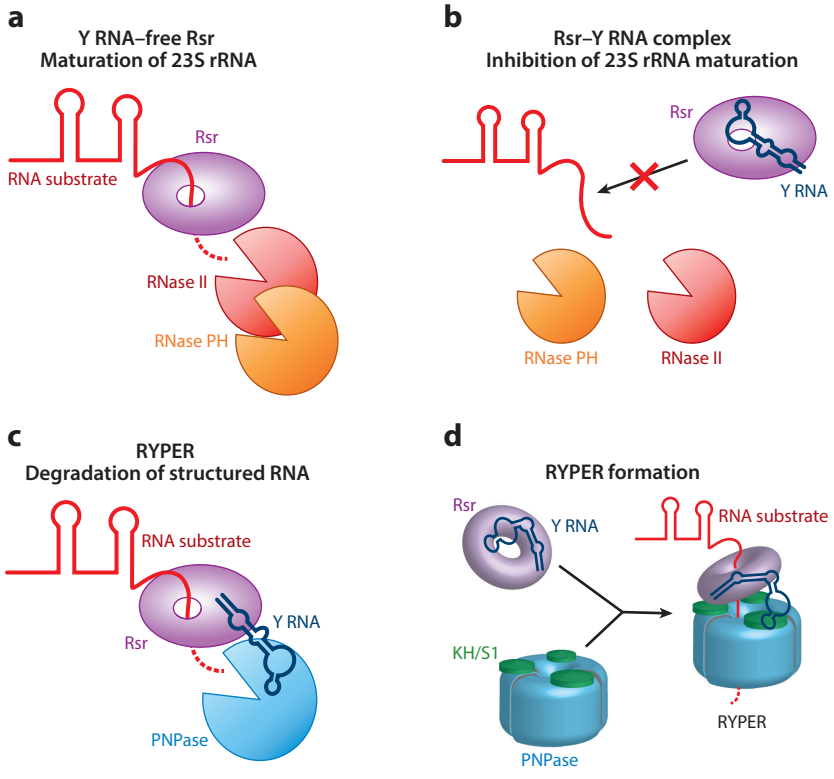


Figure 6

Functions of Rsr and Y RNAs in *Deinococcus radiodurans*. (*a*) Maturation of 23S rRNA. During growth at 37°C, a form of heat stress, the Y RNA–free form of Rsr and two exoribonucleases, RNase II and RNase PH, are required for the efficient maturation of pre–23S rRNA. (*b*) During normal growth (30°C), binding of Yrn1 RNA to Rsr results in inefficient pre–23S RNA maturation. (*c*) Rsr is tethered by Y RNA to PNPase, enhancing its ability to degrade structured RNA. (*d*) In RYPER, Rsr binds to the Yrn1 stem, while loops at the other end of the RNA contact the KH/S1 domains (*green*) of PNPase. Abbreviation: RYPER, Rsr/Y RNA/PNPase exoribonuclease RNP.

single-stranded RNA end to initiate decay and stalls on encountering stem loops (21, 23, 101). In *E. coli*, PNPase is part of the degradosome, which is scaffolded by the endonuclease RNase E and contains an RNA helicase and enolase, a metabolic enzyme (40). RNase E generates 3′ ends for PNPase to initiate decay, the helicase unwinds RNA, and enolase may enhance binding of RNase E to some substrates (13, 40). Although the exact composition varies among RNase E–containing bacteria, most degradosomes contain PNPase and a helicase (40, 107). In gram-positive bacteria that lack RNase E and instead use the endonuclease RNase Y to initiate RNA decay (63, 94), a stable degradosome has not been purified. However, PNPase copurifies with the CshA RNA helicase in *Bacillus subtilis* (62) and *Staphylococcus aureus* (39), suggesting CshA functions with PNPase to degrade structured RNA. In *E. coli*, PNPase also functions with poly(A) polymerase, which adds an A tail to the 3′ ends of structured mRNAs and ncRNAs, allowing PNPase to initiate decay (25, 66, 68, 121). Although dedicated poly(A) polymerases are confined to beta-, gamma-, and deltaproteobacteria (53), the ability of PNPase to add polynucleotide tails to its own substrates under low-phosphate conditions may serve a similar role in other bacteria (72). Y RNA–mediated

tethering of Rsr enhances the ability of PNPase to degrade structured RNA (21); this is yet another way in which the specificity of PNPase can be altered by accessory proteins.

Similar to the degradosome (40, 107), the composition of RYPER appears to vary between bacteria. Although PNPase, Rsr, and YrlA RNA copurify in *S.* Typhimurium, they sediment at a larger size (~650 kDa to 1 MDa) than *D. radiodurans* RYPER (~400 kDa) (21). Whether the stoichiometry of PNPase, Rsr, and YrlA RNA differ in *S.* Typhimurium RYPER, or whether the extra mass reflects the presence of additional components, is unknown.

Other Roles for Rsr and Y RNAs May Be Forthcoming

Although several roles for Rsr and its associated Y RNAs have been elucidated, it is likely they function in additional processes. The tRNA-like module of YrlA is conserved across most bacterial phyla (14, 19); yet, no role has been assigned to this domain. Similarly, all bacteria in which Y RNAs have been identified biochemically (as opposed to bioinformatic approaches) contain at least one Y RNA in which the effector-binding domain consists of an internal loop with a single stem loop emanating from it (**Figure 2*b*,*c***) (19, 21). The function of this RNA is unknown. Finally, since Y RNAs have eluded bioinformatic identification in some Rsr-containing bacteria (19, 43), more Y RNAs remain to be discovered.

COMPARISON OF Rsr AND Hfq

Rsr is similar in size and overall shape to the RNA chaperone Hfq, which like Rsr, has multiple roles in posttranscriptional gene regulation, particularly in response to environmental stress (29, 50, 92). Hfq contains six copies of an ~10-kDa monomer that is a member of the Sm-like protein family (73, 93, 124). As a homohexamer, the Hfq ring is symmetric (73, 93, 124), while the monomeric Ro60/Rsr ring resembles a squished donut (89, 103) (**Figure 1**).

Although some roles of Rsr and Hfq appear distinct, others are conceptually similar. The best-known role of Hfq is to stabilize sRNAs and promote their base-pairing with mRNA targets (55, 73, 92, 110, 124). While Rsr stabilizes Y RNAs (18, 19), there is currently no evidence that Rsr promotes sRNA stability or annealing.

Other roles of Rsr and Hfq appear more analogous. Although the ways in which Rsr and Hfq select their target RNAs differ, the role of Rsr in specializing PNPase for structured RNA decay resembles that of Hfq in specializing RNase E to cleave mRNAs targeted for decay by sRNAs. Hfq associates via sRNAs with the C terminus of RNase E (13, 75), forming a complex in which the 5′ monophosphate of the base-paired sRNA activates mRNA cleavage by RNase E (6). Although Y RNA–mediated tethering of Rsr to PNPase increases exonucleolytic decay of structured RNA (21), and the sRNA-mediated association of Hfq with RNase E assists endonucleolytic cleavage of single-stranded RNA (5, 75), both Rsr and Hfq act as adaptors to enhance decay of specific RNAs. RYPER also bears some resemblance to a complex in which Hfq and PNPase associate via sRNAs (7, 15). It was proposed that after RNase E cleaves the mRNA downstream of the region base-paired with sRNA, PNPase may degrade the resulting mRNA fragments (15). In this case, Hfq and its associated sRNA would resemble Rsr and Y RNA in regulating access of substrates to PNPase (15).

Other roles of Hfq, such as unfolding structured mRNAs (48) and facilitating correct maturation of pre-16S rRNA (4), are reminiscent of the roles of Rsr in assisting structured RNA unwinding (21) and pre–23S rRNA maturation (22). Although the mechanisms by which Rsr and Hfq destabilize RNA structure likely differ (since single-stranded RNA inserts through the Ro60/Rsr cavity but does not traverse the Hfq cavity), they could have overlapping functions in cells. Interestingly, some bacteria that lack a recognizable Hfq (111) contain Rsr. These

bacteria include some members of the *Deinococcus-Thermus* phylum, including *D. radiodurans*; actinobacteria such as *M. smegmatis* and *Streptomyces coelicolor*; and deltaproteobacteria such as *Sorangium cellulosum*. It will also be interesting to study Rsr in bacteria, such as *S.* Typhimurium, that contain both Rsr (21) and a well-studied Hfq (51, 115).

LOOKING FORWARD

Although studies of Rsr and Y RNAs in *D. radiodurans* revealed roles for these RNPs in assisting and regulating the degradation of structured RNAs by 3′-to-5′ exoribonucleases, we are certain that these RNPs function in additional processes. Several bacterial Y RNAs have yet to be assigned functions, and the finding that Rsr and Y RNAs are often encoded adjacent to an RNA ligase hints at a role for these RNPs in RNA repair. Biochemical and genetic studies of Rsr and Y RNA in other genetically manipulatable bacteria, such as *S.* Typhimurium, *M. smegmatis*, and *S. coelicolor*, will undoubtedly unveil new ways in which these RNPs function. Another proposed role, which we have discussed elsewhere (11), is in the etiology of those autoimmune diseases, such as systemic lupus erythematosus and Sjögren's syndrome, that are characterized by anti-Ro60 autoantibodies. If, as proposed (43), commensal Rsr RNPs trigger an immune response that, in genetically susceptible patients, subsequently targets their own Ro60 RNPs, bacterial Rsr RNPs could be therapeutic targets.

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