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Emerging roles for the Ro 60 kDa autoantigen in noncoding RNA metabolism

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Abstract

All cells contain an enormous variety of ribonucleoprotein complexes that function in diverse processes. Although the mechanisms by which many of these RNPs contribute to cell metabolism are well understood, the roles of others are only now beginning to be revealed. A member of this latter category, the Ro 60 kDa protein and its associated noncoding Y RNAs, was discovered because the protein component is a frequent target of the autoimmune response in patients with the rheumatic diseases systemic lupus erythematosus and Sjögren's syndrome. Recent studies have shown that Ro is ring-shaped, binds the single-stranded ends of misfolded noncoding RNAs in its central cavity, and may function in noncoding RNA quality control. Although Ro is not present in yeast, many bacterial genomes contain potential Ro orthologs. In the radiation-resistant eubacterium *Deinococcus radiodurans*, the Ro ortholog functions with exoribonucleases during stress-induced changes in RNA metabolism. Moreover, in both *D. radiodurans* and animal cells, Ro is involved in the response to multiple types of environmental stress. Finally, Y RNAs can influence the subcellular location of Ro, inhibit access of the central cavity to other RNAs and may also act as binding sites for proteins that influence Ro function.

Keywords

RNA quality control; Environmental stress; Y RNAs; Ribonucleoprotein complex

INTRODUCTION

The Ro 60 kDa autoantigen is a ring-shaped RNA-binding protein that is a clinically important target of the immune response in patients with two rheumatic diseases, systemic lupus erythematosus and Sjögren's syndrome^{1–4}. First characterized in humans as a 60 kDa protein that binds ~100 nt noncoding RNAs called Y RNAs^{5–10}, Ro orthologs and Y RNAs have now been described in a variety of animal and bacterial species^{11–21}. Because Ro associates with misfolded noncoding RNAs in some vertebrate nuclei, it is proposed to function in RNA quality control^{22,23}. Consistent with a role in targeting defective RNAs for degradation, a bacterial Ro ortholog functions with 3' to 5' exonucleases in stress-induced RNA maturation and degradation events^{24,25}. As animal Ro proteins are also involved in the response to several forms of environmental stress, Ro may have a conserved role in modulating RNA metabolism during stress^{19,23,26,27}. Although the functions of the Y RNAs were long mysterious, recent studies have revealed that Y RNAs can influence Ro function by regulating its subcellular distribution, inhibiting access of other RNAs to the Ro central cavity, and providing binding sites for additional proteins^{24,27,28}. In this review, we provide an overview of the current knowledge on the roles of Ro and its associated Y RNAs and discuss likely directions for further studies.

RO AS A HUMAN AUTOANTIGEN

The Ro 60 kDa protein was discovered because it is a common target of the immune response in patients with systemic lupus erythematosus and Sjögren's syndrome^{29,30}. Although the prevalence of anti-Ro antibodies in lupus patients is between 40–60%, these antibodies are detected in up to 90% of patients with Sjögren's syndrome, a chronic autoimmune disease characterized by focal lymphocytic infiltration of exocrine glands, classically the lacrimal and salivary glands^{31,32}. Most patients with anti-Ro antibodies possess antibodies against both the Ro 60 kDa protein (also called Ro60) and a structurally unrelated E3 ubiquitin ligase, TRIM21 (also known as Ro52). Although the TRIM21/Ro52 protein was initially proposed to be part of the Ro/Y RNA complex^{33,34}, subsequent studies failed to confirm the association^{35,36} and it remains unclear why autoantibodies to these two proteins are so closely linked.

Anti-Ro antibodies are of clinical interest both because of their use as a diagnostic tool and because they are strongly associated with, and may contribute to, some disease sequelae. Because of their prevalence in Sjögren's syndrome patients, the presence of these antibodies is a major criterion for diagnosis³⁷. Additionally, anti-Ro antibodies are present in >85% of mothers who give birth to infants with neonatal lupus, an autoimmune syndrome in which babies are born with permanent heart defects (classically a cardiac conduction defect, third degree heart block) and/or transient skin rashes that disappear when the maternal antibodies are no longer detected in the infant's circulation^{38,39}. In adults, anti-Ro antibodies are present in nearly all patients with subacute cutaneous lupus erythematosus (SCLE), a form of cutaneous lupus strongly associated with sun exposure^{31,40}. Notably, the skin lesions in SCLE are histologically identical to those of neonatal lupus⁴¹. However, because the majority of infants exposed to maternal anti-Ro antibodies are unaffected and many adults with anti-Ro antibodies do not develop SCLE, the antibodies may be necessary but not sufficient to confer symptoms⁴². Consistent with a requirement for additional factors, SCLE is also associated with genetic polymorphisms that result in increased production and decreased clearance of apoptotic cells⁴³.

Interestingly, the Ro 60 kDa protein and its associated RNAs were recently proposed to play a central role in the development of heart block in fetuses exposed to maternal anti-Ro antibodies⁴⁴. These studies built upon a large body of experiments that indicate that the nucleic acid-containing autoantigens that are hallmarks of diseases such as lupus are critical participants in the associated tissue damage (reviewed by 45,46). Specifically, after release from dying cells, the autoantigens form immune complexes with the patient autoantibodies that are then endocytosed by B cell receptor or Fc γ -receptor-bearing cells. Binding of the nucleic acid moieties of the autoantigens to endosomal toll-like receptors such as TLR7, which recognizes single-stranded RNA, and TLR9, which recognizes CpG-containing DNA, activates the type I interferon pathway and triggers secretion of proinflammatory cytokines, resulting in tissue damage^{45,46}. Because many types of autoantibodies, including anti-DNA and anti-Sm (which recognizes the Sm proteins of the spliceosomal U snRNAs⁴⁷) can be present in lupus patients, it is generally not known which autoantigens contribute most strongly to the disease pathology. However, because maternal anti-Ro antibodies are nearly always detected in babies with autoimmune heart block, it has been proposed that the cardiac disease is initiated when the maternal antibodies form immune complexes with Ro RNPs released by dying cardiomyocytes⁴⁴. In this model, after uptake of the immune complexes by macrophages, Ro-bound RNAs activate TLR7, triggering an inflammatory cascade that results in heart block⁴⁴. Should this model prove to be correct, it would provide a molecular explanation for the striking association of the antibodies with congenital heart block, which was first noted nearly thirty years ago^{48–50}. This model might also provide a

paradigm for determining whether anti-Ro antibodies contribute to the neonatal and adult skin lesions and/or some of the manifestations of Sjögren's syndrome.

EVIDENCE THAT ANIMAL RO 60 kDa PROTEINS FUNCTION IN NONCODING RNA QUALITY CONTROL

Ro binds misfolded pre-5S rRNAs in *Xenopus laevis* oocytes

The first evidence that the Ro 60 kDa protein bound misfolded RNAs came from experiments in which immunoprecipitations were performed from *X. laevis* ovary extracts²². These studies revealed that Ro associated with a large collection of variant pre-5S rRNAs in oocyte nuclei. To synthesize the large numbers of ribosomes needed for early development, *X. laevis* contains at least 20,000 copies of the major oocyte-specific 5S genes, and the individual members of this large multigene family exhibit significant sequence heterogeneity⁵¹. The variant 5S rRNAs bound by Ro contained approximately 8 additional gene-encoded 3' nucleotides, most likely due to the failure of RNA polymerase III to terminate at the first of three transcription termination signals. In addition, all Ro-bound RNAs contained one or more nucleotide changes from the consensus oocyte-specific 5S rRNA sequence. These nucleotide changes destabilized conserved helices and/or favored formation of alternative structure(s)^{22,52,53}. Consistent with adoption of nonfunctional structures, the variant RNAs were not detected in mature ribosomes²². Moreover, while both the precursor and mature forms of the wild-type 5S rRNA readily formed cytoplasmic storage complexes with the 5S rRNA-specific transcription factor TFIIIA, binding of TFIIIA to the mutant 5S rRNAs was inefficient. Transcriptional pulse-chase experiments revealed that the variant pre-5S rRNAs, unlike the wild-type pre-5S rRNA, were inefficiently processed to mature 5S rRNA and were instead degraded²².

How might Ro binding influence the fate of the misfolded pre-5S rRNAs? Since a bacterial Ro ortholog functions with exoribonucleases in rRNA maturation and decay (see below), one possibility is that Ro recruits exonucleases that degrade the defective RNAs. An alternative but not exclusive possibility is that Ro binding might sequester defective pre-5S rRNAs, thus preventing incorporation into the large ribosomal subunit (Figure 1). Consistent with this idea, *C. elegans* carrying a transposable element that disrupts *rop-1*, which encodes the Ro ortholog, have equivalent numbers of mutant 5S rRNAs when total RNA is sequenced, but have nearly five-fold more mutant RNAs when 5S rRNA is extracted from ribosomes⁵⁴.

Ro binds variant U2 snRNAs in mouse embryonic stem cells

The role of Ro in binding defective newly synthesized noncoding RNAs may be general, as Ro also associates with a large number of variant U2 snRNAs in mouse embryonic stem cells²³. Similar to the findings with *X. laevis* pre-5S rRNAs, microinjection of wild-type and variant mouse U2 genes into *X. laevis* oocytes revealed that association with Ro took place in nuclei, that Ro preferentially bound newly synthesized U2 snRNA precursors containing 3' extensions, and that Ro binding required formation of an abnormal helix in the variant U2 snRNAs²³. It should be noted that in these early experiments, the ability to detect Ro-bound RNAs was restricted to abundant RNAs that could be resolved in gels. Consistent with this limitation, *X. laevis* contains many variant 5S rRNA genes^{51,55}, while mouse ES cells express many variant U2 snRNAs²³. The application of sensitive new *in vivo* cross-linking, immunoprecipitation and high throughput sequencing techniques^{56–58} to identify Ro-bound RNAs should greatly expand the roster of Ro targets. Notably, using *in vivo* cross-linking followed by immunoprecipitation and conventional sequencing of cDNAs⁵⁹, rRNAs were shown to be targets of the Ro ortholog in the eubacterium *Deinococcus radiodurans*²⁵.

Ro may scavenge newly synthesized RNAs that fail to assemble with their correct RNA-binding proteins

Biochemical and structural studies have revealed how Ro recognizes misfolded pre-5S rRNAs⁵³. Binding requires that the RNA possess both a single-stranded 3' end of at least five nucleotides and one or more helices adjacent to the tail. Although the sequences of the helices are largely unimportant, Ro has some preference for uridines in the tail⁵³. Interestingly, the purified Ro also binds wild-type, presumably correctly folded pre-5S rRNA, albeit with reduced affinity⁵³, but does not interact when binding is assayed in *Xenopus* oocytes or extracts²², suggesting that specific 5S rRNA-binding proteins, such as ribosomal protein L5 and TFIIIA, normally compete with Ro. Consistent with this hypothesis, purified Ro binds other RNAs that contain both a single-stranded 3' end and adjacent helices⁵³. Together, these findings suggested that rather than recognizing a specific feature unique to misfolded RNAs, Ro may scavenge RNAs that because they are misfolded, fail to undergo 3' maturation and/or cannot be bound by their correct RNA binding proteins⁵³ (Figure 1). As expected for a scavenger, Ro binds misfolded pre-5S rRNAs with an affinity of ~25 nM, while the affinities of specific noncoding RNA-binding proteins for their targets are usually less than 5 nM^{53,60–63}. Since single-stranded uridine-rich tails are frequent features of noncoding RNA precursors, Ro could potentially contribute to surveillance pathways for many different RNAs.

The 3' ends of misfolded noncoding RNAs insert through the Ro cavity while a helix contacts the outer surface

Crystallographic studies revealed how Ro recognizes misfolded noncoding RNA precursors. The N-terminal two-thirds of Ro consists of α -helical HEAT repeats which form a ring that is closed by a von Willebrand Factor A (vWFA) domain (Figure 2A)⁶². The central cavity is lined with basic amino acids and a large basic platform abuts the cavity on the Ro surface. A co-crystal with a fragment of misfolded pre-5S rRNA revealed that the single-stranded tail inserts through the central cavity, while a helix binds the basic outer surface (Figure 2B)⁵³. Most contacts between Ro and the helix are to the RNA backbone, explaining the lack of sequence specificity and also suggesting that Ro may be able to recognize many different structured RNAs. The Ro surface has some flexibility, as several surface loops assume different conformations in three separate structures of *X. laevis* Ro^{53,62}. Additionally, a structure of the Ro ortholog from the radiation-resistant eubacterium *Deinococcus radiodurans* shows rearrangements in the conformation of several helices, a larger central cavity, and differences in the orientation of the VWFA domain, which could represent flexibility and/or differences between animal and prokaryotic Ro proteins⁶⁴ (Figure 2C).

RO MAY BE PART OF A CONSERVED PATHWAY FOR MODULATING RNA METABOLISM DURING STRESS

Although a recognizable Ro protein has not been described in budding yeast, fission yeast or protozoans, the unicellular choanoflagellate *Monosiga brevicollis* and the green algae *Chlamydomonas reinhardtii* and *Volvox carteri* all encode likely Ro orthologs, as do ~5% of sequenced bacterial genomes and several mycobacteriophages (Figure 3 and Table 1). Bacteria containing a potential Ro ortholog include *D. radiodurans*, as well as some clinically important pathogens, such as *Salmonella typhimurium*, which causes gastroenteritis, and *Bacteroides thetaiotaomicron*, a common cause of wound infections and bacteremia. Whether Ro was originally present in all bacteria, or whether the bacterial orthologs originated through multiple episodes of lateral gene transfer, is unknown.

Studies of the Ro ortholog in *D. radiodurans* have revealed that Ro is important for cell survival following certain types of environmental stress. *D. radiodurans* lacking the Ro

ortholog Rsr (Ro sixty-related) are more sensitive to ultraviolet irradiation, but not to γ -irradiation, than wild-type cells and are at a competitive disadvantage during growth in stationary phase^{19,25}. Consistent with a general role in stress metabolism, Rsr and the characterized *D. radiodurans* Y RNA are upregulated following UV, ionizing radiation and desiccation, as well as during heat stress and stationary phase^{19,24,25,65}.

Animal cell Ro proteins are also involved in the response of cells to environmental stress. In mouse embryonic stem cells and keratinocytes, Ro contributes to survival after UV irradiation^{23,66}. After either UV irradiation or oxidative stress, Ro undergoes dramatic alterations in its subcellular distribution, changing from mostly cytoplasmic to predominantly nuclear within 3 hours^{23,27}. Additionally, *C. elegans* lacking the Ro ortholog fail to form dauer larvae, a stress-resistant alternative larval stage that developing worms enter when conditions are unfavorable for growth²⁶. Finally, although the characterization of Ro orthologs in simple eukaryotes or other prokaryotes has not yet been reported, we note that these organisms include many photosynthetic species, such as the two currently available green algae genomes and many cyanobacteria (Figure 3 and Table 1). One possibility is that sunlight-requiring organisms have a greater need for pathways that increase UV resistance.

A bacterial Ro contributes to ribosomal RNA maturation and decay during stress

Studies in *D. radiodurans* have revealed that the Ro ortholog Rsr (Ro sixty-related) functions with 3' to 5' exoribonucleases to modulate RNA metabolism during some forms of environmental stress. Specifically, Rsr functions with exoribonucleases to mature 23S rRNA during heat stress and to degrade rRNA during prolonged growth in stationary phase, a form of nutritional stress. In *D. radiodurans*, 23S rRNA maturation is inefficient during normal growth at 30°C, with ~ 40% of the rRNA at steady state containing both 5' and 3' extensions²⁴. At 37°C, which results in heat stress⁶⁷, maturation becomes nearly 100% efficient and requires Rsr and the exoribonucleases RNase II and RNase PH²⁴ (Figure 4A). Additionally, both Rsr and the exoribonuclease polynucleotide phosphorylase (PNPase) contribute to the extensive rRNA degradation that occurs during stationary phase-induced starvation (Figure 4B)²⁵. Although a small fraction of Rsr and PNPase form a complex in logarithmic phase cells, complex formation increases in stationary phase²⁵. Most strikingly, the sedimentation of PNPase with ribosomal subunits increases in stationary phase and requires Rsr, suggesting that Rsr may act, directly or indirectly, to increase the association with PNPase with some RNA targets²⁵. Finally, although cells lacking PNPase are sensitive to temperature extremes, UV light, and oxidative stress, deletion of Rsr in Δpnp cells alleviates the growth defects²⁴. These genetic interactions, coupled with the finding that Rsr and PNPase form a complex^{24,25}, are consistent with a model in which the Rsr/PNPase complex also contributes to RNA metabolism during these other forms of stress. In this scenario, a molecular explanation for the observed genetic interactions could be that binding by Rsr to certain RNA targets in Δpnp cells alters their metabolism in a way that is detrimental to cell growth.

NONCODING Y RNAS REGULATE THE SUBCELLULAR LOCATION AND FUNCTION OF RO PROTEINS

In all characterized species containing a Ro 60 kDa protein, Ro associates with noncoding Y RNAs^{5,6,11,13,14,17,19}. Although these ~ 100 nt RNAs share little primary sequence homology, all Y RNAs can be folded into secondary structures containing a pyrimidine-rich internal loop and a long stem formed by base-pairing the 5' and 3' ends^{17–19,68–71} (Figure 5). Within this stem is a conserved bulged helix that is the major site of interaction with Ro^{72,73}. Structural studies reveal that a Y RNA fragment containing this high affinity site

binds to the outside of the Ro ring, with conserved amino acids in Ro contacting invariant nucleotides in the major groove of the helix⁶² (Figure 2D). In cells lacking Ro, Y RNA levels decrease by ~30-fold^{54,66}, suggesting that Ro binding protects Y RNAs from degradation.

Recent studies have revealed that Y RNAs regulate the subcellular location and function of Ro. In mouse cells, Y RNA binding blocks a nuclear accumulation signal on Ro, thus retaining Ro in the cytoplasm²⁷. In addition, structural and biochemical studies indicate that Y RNAs can sterically block access of other RNAs to the Ro central cavity^{53,62,73}.

Consistent with a role in inhibiting Ro function, studies of *D. radiodurans* Rsr have shown that the free protein functions in 23S rRNA maturation during heat stress and that the identified Y RNA inhibits maturation²⁴. These findings imply the existence of a mechanism to either dissociate Y RNAs or to reposition them on the Ro surface. Although the signals that modulate Y RNA binding remain unknown, several post-translational modifications have been identified on human Ro that could potentially influence RNA binding^{74–77}. Alternatively, binding of unidentified proteins to the large internal loop of a Y RNA could result in the repositioning of the RNA on Ro, allowing the nuclear accumulation sequence to become accessible and/or permitting other RNAs to access the cavity. Finally, most characterized vWFA domains are divalent cation-dependent protein binding sites, and in integrins the binding of ligand to this domain communicates conformational changes at distant sites⁷⁸. Similarly, binding of a protein partner to the Ro vWFA domain could result in structural changes that influence either the affinity of Ro for Y RNAs or their position on the Ro surface.

Interestingly, some species have up to four different Y RNAs^{20,21}. For example, mice have two Y RNAs (mY1 and mY3), while humans have four (hY1, hY3, hY4 and hY5). At least some of these Ro/Y RNA complexes may have functionally distinct roles. Although both mouse Y RNAs contribute to cytoplasmic retention of Ro²⁷, the human Ro/Y RNA complex containing hY5 RNA is predominantly nuclear⁷⁹. There is also considerable evidence for proteins that bind distinct subsets of the four human Ro/Y RNA complexes. For example, small fractions of the RNA-binding protein Puf60 and the ribosomal protein L5 co-purify with an exogenously expressed epitope-tagged hY5 RNA²⁸ and Puf60 also specifically interacts with the Ro/hY5 complex in a yeast three-hybrid system⁸⁰. Similarly, the RNA-binding proteins nucleolin and hnRNP I interact with a small fraction of hY1 and hY3-containing Ro RNPs but not with Ro RNPs containing hY4 or hY5 RNA^{81,82}. Finally, the zipcode binding protein ZBP1 interacts with Y3 but not other Y RNAs in both human and mouse cells⁸³ (also our unpublished data). In each case, the proteins interact with the large internal loops of the specific Y RNAs. Although the significance of these interactions have yet to be established, it has been proposed that misfolded pre-5S rRNAs are bound by L5 and that their association with Ro is enhanced by the presence of hY5 RNA²⁸.

HOW MIGHT RO INTERFACE WITH OTHER DEGRADATION PATHWAYS?

The findings that Ro associates with misfolded, defective RNAs in vertebrate cells and that a bacterial Ro functions with 3' to 5' exonucleases to mature 23S rRNA and to degrade 16S and 23S rRNAs raise the question of how the Ro pathway interfaces with other RNA maturation and decay pathways, such as those involving the eukaryotic exosome and its ring-shaped prokaryotic orthologs PNPase and RNase PH. Notably, the exosome has been most extensively characterized in *S. cerevisiae*, while PNPase and RNase PH have been best characterized in *E. coli*, two species that lack recognizable Ro orthologs.

For the *D. radiodurans* Ro, which functions with PNPase in rRNA degradation and is important for the sedimentation of PNPase with ribosomal subunits during starvation²⁵, we

speculate that Ro may function as a cofactor to assist decay of some RNA targets by PNPase. Like most 3' to 5' exonucleases, PNPase requires a 3' single stranded tail to initiate decay and has difficulty degrading structured RNA. In *E. coli*, degradation of structured RNAs is enhanced by poly(A) polymerase, which adds a 3' tail to RNAs destined for decay, and by the RNA helicase RhlB. However, most bacteria, including *D. radiodurans*, lack a dedicated poly(A) polymerase and in some of these bacteria short polynucleotide tails are added by PNPase to decay intermediates⁸⁴. Since *X. laevis* Ro binds RNAs containing a 5 nt overhang⁵³, while *E. coli* PNPase requires 7–10 nt⁸⁵, one possibility is that *D. radiodurans* Ro functions as an adapter to allow PNPase to degrade a wider variety of RNA substrates. An alternative but not exclusive possibility is that by transiently destabilizing helices adjacent to single-stranded ends, Ro could increase the processivity of associated exonucleases.

Although Ro has not been shown to associate with exonucleases in animal cells, the findings that the misfolded pre-5S rRNAs bound by *X. laevis* Ro are unstable²² and that the 3' ends of these RNAs insert through the Ro central cavity⁵³, suggest that Ro may function with exonucleases in RNA decay as it does in *D. radiodurans*. If so, we favor a model in which Ro functions in a pathway that is distinct from the exosome pathway, yet may have partially overlapping substrates. Although phosphorolytic RNA degradation occurs inside the central cavity in bacterial PNPase and the archaeal exosome, the eukaryotic exosome differs in that six catalytically inactive subunits form a hexameric ring that is topped by three subunits with RNA-binding motifs⁸⁶. In budding yeast, single-stranded RNA threads through the 9 subunit ring to reach a tenth subunit, the 3' to 5' hydrolytic exoribonuclease Rrp44/Dis3^{87,88}. It has been proposed that the catalytically inactive nine subunit ring of the eukaryotic exosome acts as a filter to ensure that only protein-free single-stranded ends are presented to the catalytic subunit for decay⁸⁸. Since Ro is also a catalytically inactive ring that binds protein-free single-stranded ends in its central cavity⁵³, Ro could function similarly to channel single-stranded RNA to exonucleases. It is also possible that Ro channels RNA to other enzymes, such as helicases.

Finally, the fact that mammalian cells lacking Ro are sensitive to UV irradiation, together with the dramatic accumulation of Ro in nuclei that occurs after UV irradiation and during oxidative stress, suggests that as in bacteria, Ro could be important in animal cells for adapting RNA metabolism in response to environmental stress. The use of *in vivo* crosslinking technologies to identify both the RNA targets of Ro and its interacting proteins under multiple stress conditions may be informative for elucidating both the range of processes affected by Ro and its precise mechanism of function.

Conclusion

Recent studies have revealed that Ro is a ring-shaped RNA binding protein that is important in the response to environmental stress in both animal cells and bacteria. The finding that Ro binds RNAs that contain both single-stranded 3' ends and adjacent protein-free helices has resulted in a model in which Ro scavenges newly synthesized RNAs that fail to associate with their correct binding proteins. Moreover, the observations that the single-stranded 3' ends of misfolded RNAs insert through the *X. laevis* Ro central cavity, coupled with the discovery that a bacterial Ro functions with 3' to 5' exoribonucleases, suggests that binding of Ro to its RNA substrates may contribute to their decay. Challenges for the future include determining the range of pathways involving Ro in both eukaryotic cells and bacteria and identifying the protein partners, such as exonucleases and/or helicases, that function with Ro in these cells. Moreover, the finding that Y RNAs influence the subcellular location and function of Ro imply the existence of regulatory mechanisms that modulate either the affinity of Ro for these RNAs or their position on the Ro surface.

Finally, given the strong linkage of anti-Ro antibodies to specific clinical disorders, the proposed involvement of Ro-bound RNAs in autoantibody-mediated tissue damage⁴⁴, and the surprising observation that mice lacking Ro develop an autoimmune syndrome with features of systemic lupus erythematosus⁶⁶, two key goals will be to unravel the extent to which Ro-bound RNAs contributes to the manifestations of disease in humans and the mechanism(s) by which Ro contributes to preventing the development of autoimmunity in mice. Here, the collaboration of molecular and cell biologists with clinicians and immunologists could result in insights that yield potential therapeutic targets.

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Figure 1. Model for the role of Ro in pre-5S rRNA quality control

Newly synthesized pre-5S rRNA can either fold into the correct structure (A) or can misfold (B). Changes in the 5S rRNA secondary structure are depicted by bold lines. Binding by Ro could sequester the misfolded RNA, preventing ribosome incorporation, and/or recruit nucleases that degrade the RNA. The drawing is not to scale.

Figure 2. Structures of Ro proteins

(A) Domain structure of *X. laevis* Ro⁶². Ro is colored according to the rainbow, with the N-terminus in blue and the C-terminus in red. N-terminal HEAT repeats form a ring that is closed by a vWFA domain. The vWFA domain contains a divalent cation binding site known as a MIDAS (metal ion dependent adhesion site) that in integrins is a ligand binding site⁸⁹. Green sphere: Mg^{2+} from the crystallization buffer.

(B) Molecular surface representation of *X. laevis* Ro bound to a fragment of misfolded pre-5S rRNA⁵³. Basic residues on the surface are colored bright blue. The single-stranded 3' end binds in the cavity and the helix binds to a basic platform on the outer surface. Most of the interactions of Ro with the helix are to the backbone.

(C) Domain structure of *D. radiodurans* Rsr shown in the same orientation and colors as *X. laevis* Ro (A). Green sphere: Ca^{2+} from the crystallization buffer⁶⁴.

(D) Molecular surface representation of *X. laevis* Ro bound to a Y RNA fragment containing the conserved helix that is required for high affinity binding. The bulged helix binds on the outer surface of Ro. In this structure, a single stranded RNA (one of the strands used to form the Y RNA duplex) binds in the central cavity⁶².

Figure 3. Unrooted phylogenetic tree showing the relationships of putative Ro orthologs from selected species. Sequence alignments were performed using ClustalW⁹⁰ and the tree was drawn by the neighbor-joining method using Phylip⁹¹.

Figure 4. Models for the roles of *D. radiodurans* Rsr in modulating rRNA metabolism during stress

(A) During *D. radiodurans* growth at 30°C, 23S rRNA maturation by PNPase is inefficient. At 37°C, a heat stress temperature, Rsr maturation becomes efficient and requires Y RNA-free Rsr, RNase II and RNase PH²⁴.

(B) During prolonged growth in stationary phase, a form of starvation, translation becomes inefficient and the levels of free ribosomal subunits increase. Rsr recruits PNPase to ribosomal subunits, thus promoting rRNA degradation. In addition to Rsr and PNPase, at least one other nuclease participates in degradation²⁵. The models in (A) and (B) are not to scale.

Figure 5. Predicted secondary structures of Y RNAs

(A) The four human Y RNAs are shown. The conserved boxed sequences are critical for Ro recognition^{62,72,73}.

(B) Predicted structures for the identified Y RNAs in *C. elegans* and *D. radiodurans*^{17,19}.

Table 1

Some bacterial species containing Ro orthologs.

Phylum	Species	Genbank accession no.
Acidobacteria	Acidobacterium sp. MP5ACTX8	EFI58263
Actinobacteria	Amycolatopsis mediterranei Catenulispora acidiphila Cellulomonas flavigena Corynebacterium amycolatum Frankia sp. Mycobacterium smegmatis Nocardia farcinica Rhodococcus opacus Rhodococcus sp. Streptomyces avermitilis Streptomyces bingchengensis Streptomyces coelicolor Streptomyces flavogriseus Streptomyces griseoflavus Streptomyces lividans Streptomyces scabies Streptomyces violaceusniger Streptosporangium roseum Thermomonospora curvata	ADJ42761 ACU69275 ADG76507 EEB62751 EFC80177 ABK75237 BAD58810 BAH50009 ABG93898 BAC74061 ADI04905 CAB46406 ADW06315 EFL42018 EFD70002 CBG75843 EFN13962 ACZ89711 ACY96403
Bacteroidetes	Bacteroides sp. 1_1_14 Bacteroides sp. 1_1_6 Bacteroides thetaiotaomicron Chryseobacterium gleum Cytophaga hutchinsonii Kordia algicida	EFI02581 EES66596 AAO76873 EFK36987 ABG60150 EDP98294
Chloroflexi	Herpetosiphon aurantiacus	ABX03794
Cyanobacteria	Acaryochloris marina Crocospaera watsonii Cyanothece sp. (strain ATCC 51142) Cyanothece sp. (strain PCC 7424) Cyanothece sp. (strain PCC 7822) Cyanothece sp. (strain PCC 8801) Cyanothece sp. (strain PCC 8802) Cyanothece sp. CCY0110 Lyngbya aestuarii Nostoc punctiforme Synechococcus sp. (PCC 7002)	ABW27217 EAM50102 ACB50443 ACK72725 ADN13996 ACK67854 ACV02761 EAZ88260 EAW36821 ACC79652 ACB01180
Deinococcus-Thermus	Deinococcus radiodurans Meiothermus ruber Meiothermus silvanus	AAF10833 ADD27995 ADH65180
Firmicutes	Acetivibrio cellulolyticus Bacillus mycoides Bacillus pseudomycoides Bacillus thuringiensis Clostridium beijerinckii Clostridium cellulovorans Clostridium thermocellum	EFL63739 EEM08399 EEM16310 EEM25069 ABR35679 ADL53084 ABN53963
Planctomycetes	Pirellula staleyii Planctomyces brasiliensis Planctomyces limnophilus Planctomyces maris Rhodopirellula baltica	ADB15052 ADY60717 ADG66354 EDL56876 CAD79113

Phylum		Species	Genbank accession no.
Proteobacteria	Alpha-proteobacteria	Agrobacterium radiobacter Asticcacaulis excentricus Bradyrhizobium sp Methylobacterium nodulans Methylobacterium radiotolerans Methylosinus trichosporium Pseudovibrio sp. JE062 Rhizobium etli Rhodocyclidium vanniellii Roseibium sp. TrichSKD4 Roseobacter sp. SK209-2-6 Silicibacter pomeroyi	ACM28474 ADU13735 CAL77486 ACL61958 ACB23801 EFH00996 EEA96694 ACE93690 ADP71709 EFO33281 EBA18470 AAV93485
	Beta-proteobacteria	Acidovorax avenae Comamonas testosteroni Eikenella corrodens Kingella oralis Neisseria elongata Neisseria flavescens Neisseria sicca Neisseria subflava Ralstonia pickettii Uncultured beta proteobacterium HF0010_04H24	ADX43942 EED66736 EEG25246 EEP66649 EFE48372 EER55958 EET43536 EFC52289 ACD27471 ADI18848
	Gamma-proteobacteria	Beggiatoa sp. Cardiobacterium hominis Hahella chejuensis Pseudomonas fluorescens Salmonella typhimurium LT2	EDN67448 EEV88413 ABC28423 ABA73688 AAL22382
	Delta/epsilon-proteobacteria	Plesiocystis pacifica Sorangium cellulosum	EDM80164 CAN92696
Verrucomicrobia		Bacterium Ellin514 Chthoniobacter flavus Ellin428	EEF62205 EDY18771