Cell, Vol. 121, 495–501, May 20, 2005, Copyright ©2005 by Elsevier Inc.

Ro's Role in RNA Reconnaissance

The Ro 60 kDa autoantigen binds misfolded RNAs and likely functions in small RNA quality control. In this issue of *Cell*, Stein et al. (2005) present crystal structures of Ro alone and bound to both double- and single-stranded RNA, revealing two distinct RNA binding sites that suggest how Ro may distinguish between native and misfolded small RNAs.

As cells live and grow, they are faced with the challenge of making millions of copies of very complex macromolecules with high fidelity. While mechanisms underlying protein, DNA, and mRNA quality control are fairly well established, little is known about how cells cope with misfolded and otherwise abnormal noncoding RNAs. The Ro protein has attracted attention in this regard as one of the few proteins that specifically recognizes misfolded small RNAs in vivo.

Ro was originally described over twenty years ago as a major autoantigen in patients suffering from lupus erythematosus. The Ro autoantigen was identified by its association with a class of small RNAs of unknown function, termed Y RNAs (Lerner et al., 1981). The Ro 60 kDa protein has subsequently been shown to be an abundant component of virtually all vertebrate cells (Hendrick et al., 1981), and orthologs of Ro exist in the nematode *C. elegans* as well as in various unicellular eukaryotes and prokaryotes (Labbe, 1995). Despite the ubiquitous nature of this protein and intense medical interest in lupus and other autoimmune diseases, however, an understanding of the biological function of Ro and Y RNAs has only recently begun to emerge. Studies in mouse embryonic stem cells and the radiation-resistant eubacterium *D. radiodurans* revealed a conserved role for Ro in contributing to cell survival after ultraviolet irradiation (Chen et al., 2003; Chen et al., 2000). At the same time, Ro-deficient mice were found to develop lupus-like symptoms, suggesting that Ro may also be involved in preventing autoimmunity (Xue et al., 2003). So what is the link between ultraviolet resistance, autoimmunity, and the mysterious Y RNAs? Recent biochemical experiments suggest a possible answer.

In a methodical series of studies, Sandra Wolin and colleagues have previously shown that the Ro protein associates not only with Y RNAs but also with misfolded small cellular RNAs. The best characterized of these interactions is between Ro and a variant misfolded form of pre-5S rRNA, a small RNA that is a component of the large ribosomal subunit. Variant pre-5S rRNAs in *Xenopus* oocytes contain nucleotide changes that promote the formation of a helix not normally present in functional 5S rRNA, as well as extra nucleotides at the 3′ end resulting from errors in transcriptional termination (Shi et al., 1996). Similarly, Ro has been found to bind variants of small nuclear U2 RNAs that have the propensity to form an abnormal helix (Chen et al., 2003). Moreover, *C. elegans* lacking Ro have an increased proportion of variant 5S rRNA in their ribosomes (Labbe et al., 1999). Given these observations, it has been proposed that the cellular function of Ro is to act in small RNA quality control (reviewed in Chen and Wolin, 2004). This would suggest that RNAs damaged by ultraviolet irradiation may be Ro substrates and that failure to clear aberrant RNAs may expose normally cryptic determinants to the immune system.

But if Ro really is responsible for detecting, and perhaps correcting, the structures of small RNAs, how does it discriminate between misfolded and properly folded molecules, and what is the role of the Y RNAs in this process? Karin Reinisch and colleagues have now taken an important step in answering these questions by determining crystal structures of Ro in the presence and absence of a conserved duplex fragment of Y RNA.

The crystal structure of Ro shows that the protein consists of an N-terminal donut-shaped domain made up of HEAT repeats and a C-terminal von Willebrand factor A (vWFA) domain. The overall shape of the molecule is that of an elliptical toroid with a positively charged tunnel running through the center of the HEAT repeats. Stein et al. (2005) speculate that the vWFA domain may interact with other proteins of the quality-control pathway since RNA binding is restricted to the N-terminal HEAT repeat domain. The Y RNA duplex fragment binds to a highly conserved basic patch on the outer surface of the N-terminal domain. To the surprise of the researchers, an additional single-stranded RNA oligonucleotide was seen bound in the central tunnel. Since single-stranded RNA was not intentionally included in the crystallization conditions, Stein et al. speculate that one of the strands in the Y RNA duplex must have been in excess over the other and therefore remained single stranded when the RNA was annealed.

This fortuitous accident reveals an unexpected second RNA binding site on Ro and suggests a model for how Ro may associate with misfolded RNAs (Figure 1). Stein et al. propose that the abnormal helix of misfolded RNA binds to the outer surface of the N-terminal domain while the 3′ single-stranded extension, which is always present in misfolded pre-5S rRNA, is inserted into the central tunnel. Mutagenesis confirms a role for both the outer surface and central tunnel in variant pre-5S rRNA binding, while only mutations in the outer surface of the N-terminal domain affect binding of Y RNA.

The proposed model has several appealing consequences. First, it suggests a role for Y RNA as a negative regulator of Ro activity. Since the Y RNA binding site on Ro extensively overlaps with the misfolded RNA binding site, Y RNA sterically blocks access of other RNAs to Ro. Second, the apparent plasticity of the central tunnel, established by comparing the structures of Ro in the presence and absence of RNA, allows Ro to accommodate a variety of single-stranded RNA sequences. This suggests that once bound, a misfolded RNA may be threaded through the tunnel as it is processed by downstream quality-control machinery.

As is often the case with exciting breakthroughs, this

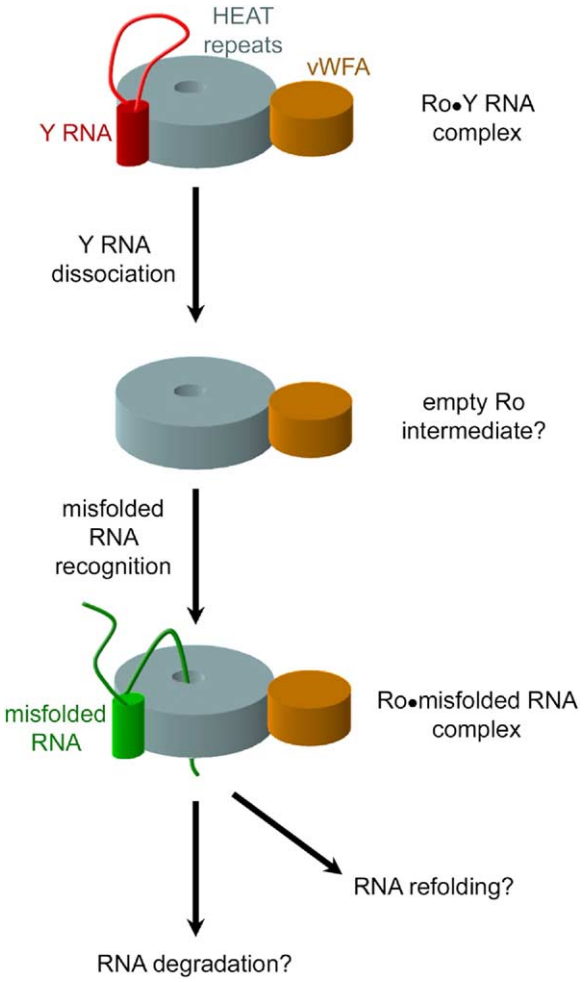

Figure 1. Possible Model of Ro Action

Y RNA sequesters Ro in an inactive state. Following an unknown trigger, the Y RNA is released and replaced with misfolded RNA, which is threaded through a central tunnel in the protein and ultimately removed by a yet-to-be-determined mechanism.

work raises as many questions as it answers. In particular, what triggers Ro to switch from binding Y RNA to misfolded RNA? And, once recognized by Ro, what is the fate of the misfolded RNA? Does Ro act as a processivity factor for the degradation of misfolded RNAs, or does it assist in destabilizing misfolded helices to facilitate refolding? The structures presented here provide a conceptual framework to begin addressing these questions, which ultimately will provide a clear picture of how cells detect and maintain the quality of small functional RNAs.

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DOI 10.1016/j.cell.2005.05.004