Investigation of Non-coding RNA Structure and Dynamics

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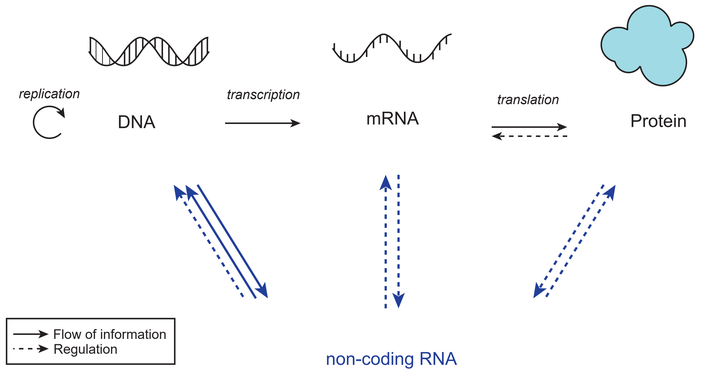
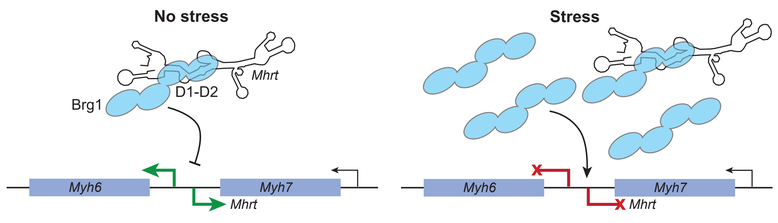
Non-coding RNAs in Biology

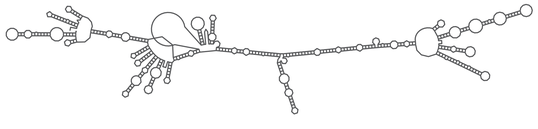


RNA can have many different roles in biology. It's best known role is as an intermediate between DNA and proteins in the form of messenger RNA. Non-coding RNAs do not play this role, but instead can act as gene regulators or catalysts for other biological functions. DNA forms well understood structures, specifically the double helix, which is made up of two complimentary strands of DNA. RNA, on the other hand, is single stranded, but can form structures within that single strand. Of particular interest are long non-coding RNAs (lncRNAs) which are generally defined as a non-coding RNA of over 200 nucleotides in length. Due to their size, these RNAs have the ability to form complex, dynamic structures, but these features are complicated to elucidate experimentally. Often, biological function is studied and determined without knowing molecular scale details about the RNA.

Mhrt, a Heart Specific lncRNA

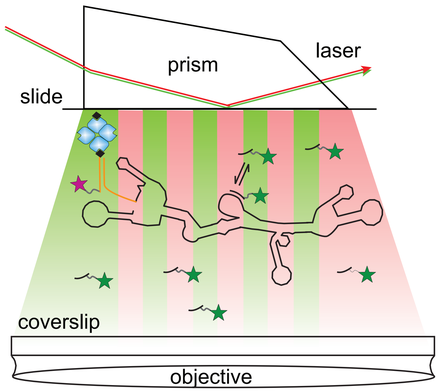


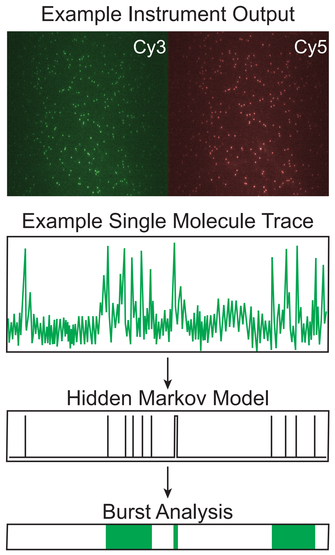
A transcript deemed *Mhrt* has been identified as a heart specific lncRNA with an observered cardioprotective function, though the exact mechanism of this function is less certain (1,2). One study outlines interactions between *Mhrt* and the chromatin remodeling factor Brg1 (1). It has been proposed that this interaction plays a role in regulating the transcription of *Myh6* vs *Myh7*, and the transcription of *Mhrt* itself. After induced stress in the heart, the restoration of *Mhrt* has improved physiological outcomes. *Mhrt* has been proposed as a potential therapeutic target, but the structure has yet to be experimentally determined. Determining this structure would provide insight into the specific mechanisms that could be targeted to improve patient outcomes.



Predicted structure of *Mhrt*

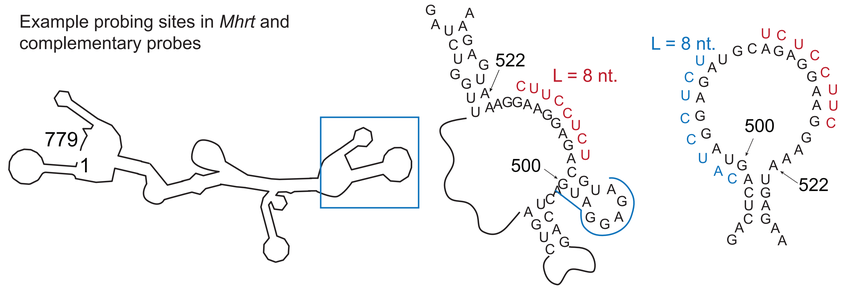
Single-molecule Structure Analysis





While many methods exist to study RNA structure, single-molecule methods are particularly advantageous for dynamic systems, as they avoid ensemble averaging. This means short lived or particularly dynamic states can be observed where they would otherwise go unnoticed. In this project, a technique called single-molecule kinetic analysis of RNA transient structure (SiM-KARTS) is used (3,4). This technique takes advantage of natural base pairing to probe regions of the RNA and determine the accessibility of that region for binding. For example, we expect to see binding and dissociation of the probe in the case that the region is single-stranded. In the case of a dynamic region, the ability of the probe to bind will change over time.

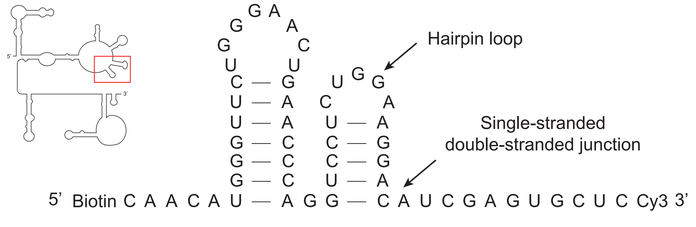
Example probing sites in *Mhrt* and complementary probes



Method Exploration

To simplify troubleshooting, and gain a deeper understanding of this technique, a model construct from the better understood lncRNA *Braveheart* was designed (5). This construct consists of multiple structures of interest, including hairpin loops and single-stranded double stranded junctions.

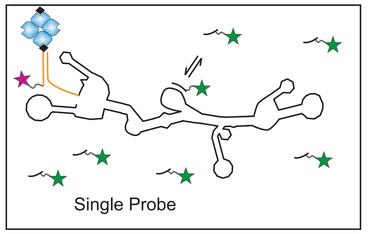
Braveheart Structure and Construct



Mhrt Experiments

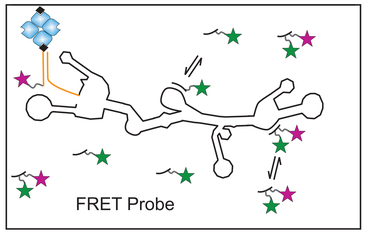
Structural Analysis using Single Probes

A single site on the RNA can be probed using a short piece of single-stranded DNA labeled with a fluorescent probe. In this case, Cy3 (green) is used. This can be repeated for different sites along the RNA.



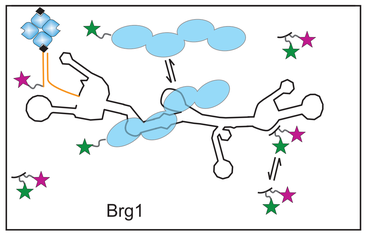
Structural Analysis using FRET Probes

Two sites on the RNA can be investigated simultaneously using two probes, one labeled with Cy3 and one with both Cy3 and Cy5 (red). If both are bound, excitation of Cy3 will result in both Cy3 and Cy5 fluorescence through a photo-physical process known as fluorescence resonance energy transfer (FRET).



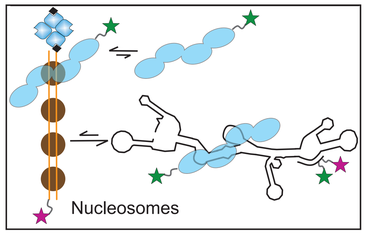
Investigation of Brg1 Binding

Using the previously described FRET probes and Cy3 labeled Brg1, the binding of Brg1 and structure of *Mhrt* can be explored. This will uncover details about biological function that have yet to be fully understood.



Exploration of Brg1 Partitioning Between Mhrt and Chromatin Bound States

Similarly, the behavior of Brg1 in the presence of both nucleosomes (DNA packaged as it would be inside the body) and *Mhrt* will be investigated using Cy3 labeled Brg1. Here, the nucleosomes are immobilized on the slide, while *Mhrt* can be located using a stably bound FRET probe.



Acknowledgments

References

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