ECLIPSEBIO
THE RNA GENOMICS COMPANY

Research Summary

Using Chemical Probing to Determine the Structure of a Viral 5′ UTR and its Function in Translational Regulation

Introduction

RNA is a multidimensional information molecule, carrying biological code within its primary, secondary and tertiary structure. Higher order structures within an RNA molecule play a crucial role in gene expression. For example, stem loops can act as molecular switches by binding specific RNA binding proteins[1] or sterically interfere with translating ribosomes[2]. The 3′ and 5′ untranslated regions (UTRs) of RNA are of particular importance, as they play a significant role in stability, export and expression control[3].

Viral 5′ UTRs are necessary for viral replication and translation and contain highly conserved secondary structures that help to regulate these processes[4]. An accurate model of viral UTR structure is necessary to understand the molecular biology of the viral life cycle. This has relevance not only in infectious disease, but also in the engineering of more potent UTRs for efficient expression of biotherapeutics, like RNA vaccines.

eSHAPE™, Eclipsebio's SHAPE (selective 2′hydroxyl acylation analyzed by primer extension) product uses NAI (2-methylnicotinic acid imidazole) for chemical probing, which offers an economical, rapid, and accurate method to empirically guide RNA folding algorithms to better predict RNA secondary structure.

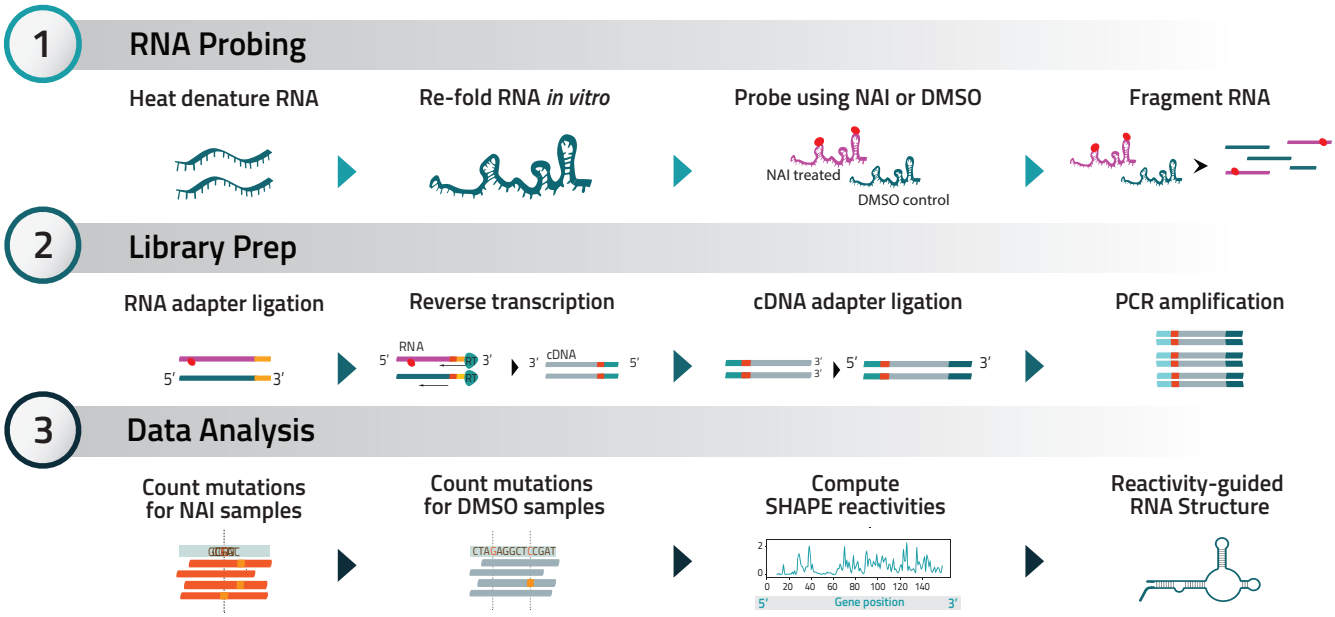

Figure 1. The Eclipsebio eSHAPE workflow. RNAs are probed with NAI and a DMSO control. NAI causes adducts to be selectively formed on free 2′-OH groups on the ribose of more flexible (unpaired) nucleotides. During reverse transcription (RT), the RT enzyme places incorrect bases opposite some of the adduct-associated nucleotides. These mutations are counted via NGS for the NAI and DMSO treated samples and base reactivities are calculated. The empirical SHAPE data is used in folding software to more accurately guide folding prediction.

Eclipsebio's eSHAPE Technique

The eSHAPE technique leverages the ability of NAI to induce chemical adducts at free 2′ OH groups on the sugar-phosphate backbone, preferentially in single-stranded regions. During subsequent reverse transcription of the NAI-modified RNA, mutations are induced at some of the bases complementary to adduct sites (Figure 1). These mutations are then captured via deep sequencing and counted in the NAI treated and DMSO control samples across transcripts. Reactivity scores for each base are calculated by subtracting background (DMSO control) from NAI mutation rates. Reactivity scores will generally be higher for unpaired positions. The normalized reactivity values can then be used in folding software (e.g., RNAStructure) to help guide the RNA folding prediction. The addition of eSHAPE reactivities into these folding algorithms renders more accurate RNA folding predictions due to the empirical nature of eSHAPE data.

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Accurate Secondary Structure Prediction of a Viral 5' UTR to Determine UTR Function

Mackeown *et al.*[5] used DMS (dimethyl sulfate)-MapSeq and NAI-based eSHAPE (Eclipsebio) to determine the average structure of the human coronavirus HCoV-OC43 5' UTR. While both methods can be used for structure prediction, NAI-based eSHAPE is superior because NAI probes all four nucleotides, while DMS probes only adenosine and cytosine.

Nevertheless, the two chemical probing methods yield very similar structures (Figure 2B and C). The major difference between the two predictions is in the structure of stem loop 5[mb], where DMS probing informed 5 sub-stem loops versus only 3 for NAI-based probing. The authors conclude that this empirical evidence supports stem loops 1-4 being more stable and the SL5[mb] domain being more dynamic. Indeed, chemical probing profiles the population of molecules in a snapshot and will represent an average structure of typically very dynamic RNA molecules. Additionally, the authors show that orthogonal structure approaches, NMR and small-angle X-ray scattering (SAXS), agree remarkably well with the eSHAPE results.

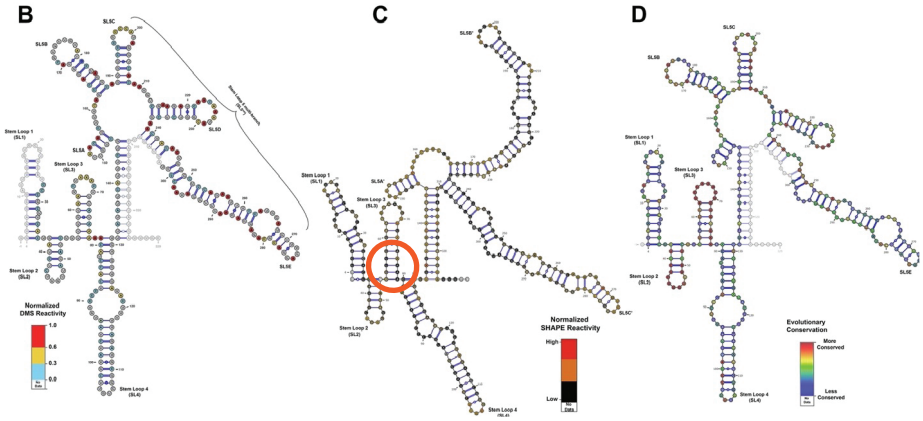

Figure 2. A reproduction of Figure 1 from Mackeown *et al.* SHAPE structures derived from DMS probing (B) and NAI probing (C). Higher reactivities are indicative of unpaired nucleotides. DMS probes adenosine and cytosine only while NAI probes all four nucleotides. Evolutionary conservation of the DMS structure (D). The orange circle has been added to the original figure to highlight the region that was mutated to disrupt the structure of the stacked helix.

Mackeown *et al.* also demonstrate functionality of the 5' UTR by way of a reporter gene assay that shows that the 5' UTR is required to repress viral translation. Importantly, removing a coaxial stacked helix in stem loops 3 and 4 by mutation (Figure 2C, red circle) significantly increases translation (by up to 167%) in a reporter assay, suggesting the role of this structure is to repress translation by sterically interfering with ribosome scanning.

"SHAPE helped to illuminate the dynamic nature of several key stem loops within the 5'UTR. Without these observations we could have missed a key regulatory mechanism for the virus," said Case Western Reserve University researcher, Matthew Mackeown, PhD.

A correct understanding of UTR function first requires an accurate understanding of UTR structure. This study demonstrates the power of the eSHAPE technique to accurately predict the secondary structure of a viral 5' UTR and to inform subsequent mutational analyses that help determine UTR function. eSHAPE is a straightforward, rapid and accurate method for RNA folding prediction and when used in combination with folding algorithms, provides a more accurate prediction of structure than computational methods alone.

References

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