Structural Features of Alu RNA that Guide ADAR-Mediated Editing

Ivana Borovska1 , Livia Pelegrinova1 , Milan Hucko2 , Lubos Klucar2 , Jana Kralovicova1
1 Institute of Molecular Physiology and Genetics, Centre of Biosciences, Slovak Academy of Sciences, Bratislava, Slovakia, 2 Institute of Molecular Biology, Slovak Academy of Sciences, Bratislava, Slovakia

Background: The diverse regulatory roles of RNA are based on the unique structural features of these molecules that are also a hallmark of the Alu elements, the most successful parasites of the human genome. They originated as retrotransposons during early primate evolution and have evolved to play key roles in gene expression, such as alternative pre-mRNA splicing or translation. Inverted Alu repeats (IRAlus), formed by neighbouring oppositely oriented Alu sequences embedded within mRNA transcripts, represent the predominant substrates for adenosine deaminases acting on RNA (ADARs). Extensive adenosine-to-inosine (A-to-I) modification plays a critical role in mRNA processing and innate immune sensing. Deregulation of A-to-I editing is associated with a range of autoimmune, oncological and neurological diseases and decreases with age. The reliance of A-to-I editing on double-stranded substrates has led to the conclusion that the interaction between IRAlus involves the formation of long intramolecular RNA duplexes. Method: We combined structure-based mutagenesis of plasmid reporters, RNA secondary structure mapping and transcriptome-wide A-to-I editing analyses to investigate how the IRAlu structure shapes ADAR-mediated editing. Results: We demonstrate that editing efficiency does not primarily reflect predicted duplex stability or sequence motifs but instead relates to the Alu's capacity to adopt a conserved ancestral structure. Furthermore, altered levels of the SRP9/14 heterodimer, which supports the closed conformation of Alu RNA, affected the extent of IRAlu modifications. Conclusion: Our study provides new insight into the relationship between IRAlu architecture and A-to-I editing, emphasising the importance of specific structural motifs and interacting proteins and establishes a foundation for future studies into the molecular mechanisms underlying IRAlu function.