Characterization of the structural and functional dynamics of active LTR retrotransposon RNA genomes

Angelika Andrzejewska-Romanowska

ABSTRACT

LTR (long terminal repeat)-retrotransposons replicate via genomic RNA (gRNA), which serves as a template for protein synthesis and reverse transcription. Similar to RNA viruses, both the sequence and structure of gRNA retrotransposons carry the instructions necessary for replication. The development of RNA structure chemical mapping methods allows us to deepen our knowledge about the native structure of RNA molecules in the cellular environment. It is necessary to obtain a comprehensive picture of the impact of RNA architecture on its function. Despite the growing number of RNA structural models, data on the *in vivo* structure of retrotransposon gRNA have not been available.

The most important goal of my research was to determine for the first time the gRNA structure of active LTR-retrotransposons during replication in cells. My research models were retrotransposons Ty1 (*Pseudoviridae*) and Ty3 (*Metaviridae*), naturally occurring in the *Saccharomyces cerevisiae* genome. The studies on yeast Ty element biology are essential sources of information about the mechanisms of retrotransposition. My doctoral dissertation consists of a series of three thematically related publications. In the work by **Andrzejewska et al.*, NAR*, 2021**, I used the SHAPE-CE method to examine the gRNA structure of the Ty1 retrotransposon (5.7 kb) *in vivo*. I also performed a comparative analysis of the obtained Ty1 gRNA model with the *in vitro* model. I characterized the structural properties of functional sequences engaged in RNA-RNA interactions significant for retrotransposition, indicating those that may occur before gRNA packaging into virus-like particles. Additionally, I conducted experiments in yeast inhibited for translation, showing the influence of ribosomes on the destabilization and reorganization of the Ty1 gRNA structure. In the publication by **Andrzejewska-Romanowska et al.*, NAR*, 2024**, I used the SHAPE-MaP strategy to determine the structure of gRNA Ty3 (5.1 kb) *in vivo* and *ex vivo.* I identified the state-specific structural motifs and a well-folded core forming independently from the cellular environment. I described the structural context of known functional sequences and proposed a novel Ty3 gRNA dimerization sequence. Finally, I characterized gRNA key structural features shared between representative retroelements (Ty1, Ty3, and HIV-1). The series begins with a review by **Andrzejewska et al.*, IJMS*, 2020**, summarizing the available knowledge about RNA structure *in vivo* resulting from the most recent studies in different organism cells. We focused on factors regulating the structure of RNA inside cells and the relationships between the structure of RNA molecules and their function.