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Characterization of RNA-binding proteins and circular RNA-protein interactions in the mouse brain

Abstract

RNA-binding proteins (RBPs) play crucial roles in regulating various aspects of RNA function within cells. Aggregation of RBPs, alterations in their subcellular localization, and disruptions in RNA-protein interactions have been linked to the pathogenesis of several neurological diseases. In recent years, RNA, including numerous non-coding RNAs (ncRNAs), has also been found to participate in the regulation of RBP function and localization. For instance, certain circular RNAs (circRNAs) have been shown to contribute to the regulation of RBP function, serving as scaffolds, protein stabilizers, or participating in their transport. Although several important circRNA-protein interactions in the nervous system have been described, the functions of most endogenous circRNAs remain largely unknown.

Despite development of many new methods for studying RNA-protein complexes, most of them have been optimized to be used in cell lines. This doctoral dissertation focused on optimizing high-throughput methods for studying RNA-protein interactions directly in tissue, to provide identification and analysis of native RNA-RBP interactions naturally occurring in a living organism. The primary aims were to characterize the RBP repertoire in the brain and to gain insights into the functions of endogenous circular RNAs within this organ.

In the first part of this work, XRNAX method was adapted to identify the total RNA-protein interactome in wild-type mouse brain tissue. Two brain homogenization methods were compared, and biopulverization was selected as the technique best preserving the integrity of RNA-protein interactions after UV irradiation. Stable RNA-protein complexes were isolated using the XRNAX procedure and further purified on a silica column, with peptides identified by mass spectrometry. Several hundred proteins were identified, the majority of which were known, canonical RBPs. Subsequently, the same procedure was applied to analyze brain tissue from mice with a myelination defect (*shiverer* mice). The results revealed disruptions in RNA-

protein interactions among canonical RBPs involved in alternative splicing and stress granule formation. These observations were confirmed for MBNL1 protein. MBNL1 was shown to be abnormally accumulated in the cell nuclei, which likely resulted in altered alternative splicing pattern among MBNL1-regulated genes. Further, the subcellular mislocalization of MBNL1 has been linked to reduced expression of *circMbnl1(3,4,5,6)*, which contains a nuclear localization signal (NLS). This coincided with the increased expression of mRNA *Mbnl1* also containing an NLS sequence. This evidence suggests that dysregulation of circRNA can affect the localization of a protein encoded by the same host gene.

In the second part, of my dissertation, I optimized circRNA pull-down protocol for isolation and analysis of proteins bound to endogenous circRNAs in the mouse brain. The efficacy of the protocol has been confirmed by evidencing the enrichment of circRNA *Cdr1as* along with its known partners: miR-7 and AGO2. Proteomic analysis also revealed novel interactions of *Cdr1as* with several other RBPs and synaptic proteins. Additionally, specific pull-down of two other brain-enriched circular RNAs, *circDtnb(5,6,7,8)* and *circHipk3(2)*, has been performed, and their protein-binding potential has been assessed. Furthermore, miR-124-3p has been identified as directly interacting with *circDtnb(5,6,7,8).* In a broader perspective, the application of this RNA pull-down method can contribute to a better understanding of the functions of many other endogenous circRNAs in the nervous system.

In summary, this dissertation presents novel high-throughput protocols for studying native RNA-protein interactions in tissues and provides new data expanding our knowledge about RNA binding proteins and circRNA functioning in the brain cells in homeostasis and in neurological pathology associated with dysmyelination.