Ayça Olcay

Department of Non-coding RNAs

Institute of Bioorganic Chemistry, Polish Academy of Sciences

Loss of Function Studies of Synapse-enriched circRNAs in Neurons

Abstract

Previous studies have shown that non-coding RNAs play important roles in nervous system, including synaptogenesis. Circular RNAs (circRNAs) are particularly abundant in the brain and have been linked to synaptic transmission and plasticity, such as Cdr1as and circHomer1a. A subset of circRNAs is enriched in synaptoneurosomes, suggesting a potential role in synaptic processes; however, the functions of most synapse-enriched circRNAs remain unknown.

The aim of this study was to investigate the potential functions of selected synapse-enriched circRNAs in mouse primary cortical neurons. Sixteen circRNAs enriched in neurons, synaptoneurosomes, and frontal cortex were initially selected based on RNA-seq data. From these, three candidates—circGigyf2(4,5,6,7,8), circDtnb(5,6,7,8), and circMyst4(2)—were prioritized for functional analysis. All three circRNAs were more highly expressed in primary neurons than in astrocytes and showed higher expression levels compared to their linear mRNA counterparts. Their back-splice junctions were verified by Sanger sequencing, and RNase R resistance confirmed their circularity.

The expression analysis revealed that the levels of all three circRNAs increased from postnatal day 0 to adulthood in the mouse cortex, suggesting developmental regulation. Loss-of-function studies in primary neurons showed that knockdown of circGigyf2(4,5,6,7,8)—but not circMyst4(2) or circDtnb(5,6,7,8)—led to a significant downregulation of *Dlg4* mRNA (encoding PSD-95), *Cadm1* mRNA (encoding SynCam1), and *Icam5* mRNA (encoding telencephalin), as well as a decrease in Synapsin-1 protein levels, indicating its potential role in synapse stability and synaptogenesis.

To further explore the molecular mechanisms underlying these effects, *in silico* analyses were performed. Conservation analysis revealed a highly conserved core region across vertebrates in exons 4–7 of circGigyf2(4,5,6,7,8), supporting its functional relevance. No significant IRES elements or miRNA interactions were detected. However, predicted interactions with the RNA-binding proteins FMRP and TDP-43- known regulators of *Dlg4* mRNA (encoding PSD-95)- suggest a potential post-transcriptional regulatory mechanism.

Altogether, this study identifies circGigyf2(4,5,6,7,8) as a developmentally regulated, synapse-enriched circRNA that may regulate synaptic gene expression through interactions with RNA-binding proteins. These findings provide the first evidence linking a synapse-enriched circRNA to the regulation of transcripts encoding PSD-95, telencephalin and SynCam1, contributing to our understanding of the molecular landscape underlying synaptic stability and synaptogenesis.