

The Rat ZNF292 Gene in Pituitary Cells Produces Related Circular RNAs with Shared Combinations of Exons

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ZNF292 (formerly Zn-16) encodes a protein that is a Growth Hormone transcription factor protein with 16 DNA-binding zinc fingers. It also has a role in tumor suppression in certain cancers and in neurodevelopmental disorders with features of autism spectrum disorder. The ZNF292 gene contains eight exons that encode the full-length linear mRNA. In human cells using RNA seq analysis, ZNF292 was found to produce a circular RNA (circRNA) by “back-splicing” of exons 2-4. To determine whether circRNA ZNF292 is present in rat cells, we used RT-PCR rather than RNA seq. CircRNA primers were designed with two constraints: unique circular junctions must be amplified, and the primers must not give productive amplification on linear ZNF292 RNA. Rat pituitary MtT/S cell RNA was reverse transcribed into DNA and the primer pairs were used for PCR amplification. These reactions gave products indicating that ZNF292 circRNA exists in the rat. Further, there were multiple product bands, indicative of multiple circular RNAs in the region of amplification. These bands were isolated and sequenced. First, alignments revealed that rat circRNA contains an alternate Exon 1A as found in human ZNF292 circRNA. Second, the multiple PCR products each contain DNA from exons 1A, 2, 3, and 4 through 5 in different “nested” combinations. To further test that these products were circRNA, linear RNA was removed using RNase R treatment prior to amplification. After linear RNA removal, nested sets of product sizes were again present in these samples, confirming circular origin. These products are currently being sequenced for complete identification. While the function of circRNAs remains unknown, ZNF292 circRNAs in rat pituitary cells are present in several related sizes ranging from small to large. These products share a common core of back-spliced exons, with additional exons added to that core. We interpret this series of circular RNAs as a nested set of babushka or “Russian Dolls” – contained within each larger circle is a smaller one. The study of how these circular RNAs are formed could be important for understanding the function of these unique RNAs, which may act as a microRNA sponge or RNA-binding protein sponge used to regulate gene expression.

Funded by the Bill Gatton College of Pharmacy.