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A precise and efficient circular RNA synthesis system based on a ribozyme derived from *Tetrahymena thermophila*

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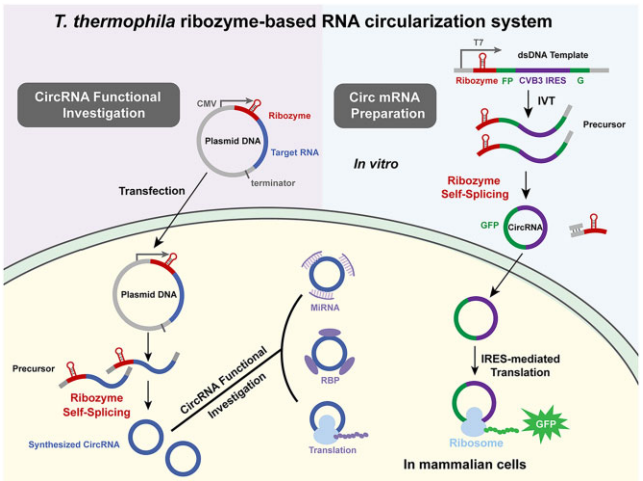
ABSTRACT

Classic strategies for circular RNA (circRNA) preparation always introduce large numbers of linear transcripts or extra nucleotides to the circularized product. In this study, we aimed to develop an efficient system for circRNA preparation based on a self-splicing ribozyme derived from an optimized *Tetrahymena thermophila* group I intron. The target RNA sequence was inserted downstream of the ribozyme and a complementary antisense region was added upstream of the ribozyme to assist cyclization. Then, we compared the circularization efficiency of ribozyme or flanking intronic complementary sequence (ICS)-mediated methods through the *DNMT1*, *CDR1as*, *FOXO3*, and *HIPK3* genes and found that the efficiency of our system was remarkably higher than that of flanking ICS-mediated method. Consequently, the circularized products mediated by ribozyme are not introduced with additional nucleotides. Meanwhile, the overexpressed circFOXO3 maintained its biological functions in regulating cell proliferation, migration, and apoptosis. Finally, a ribozyme-based circular mRNA expression system was demonstrated with a split green fluorescent protein (GFP) using an optimized Coxsackievirus B3 (CVB3) internal ribosome entry site (IRES) sequence, and this system achieved successful translation of circularized mRNA. Therefore, this novel, convenient, and rapid engineering RNA circularization system can be applied for the functional study and large-scale preparation of circular RNA in the future.

GRAPHICAL ABSTRACT



INTRODUCTION

Circular RNAs (circRNAs) are a class of covalently closed RNA molecules produced from precursor mRNA back splicing and are widely found in nature (1). Due to the lack of 5' or 3' ends, circRNAs have a certain resistance to exonuclease digestion, which makes circRNAs more stable than linear RNAs. Most natural circRNAs are noncoding, although a few of them can be translated into peptides (2,3). Noncoding circRNAs can serve as sponges and influence corresponding functions by binding to miRNAs and

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