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# Research progress on circular RNAs

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**Abstract.** Circular RNAs (circRNAs) as a new type of non-coding RNA are special circular molecules with covalently closed 3' end and 5' end. Although they were previously discovered as errors in RNA splicing process and drew little attention, their unique properties, powerful functions and as potential disease biomarker are being increasingly acknowledged by scientists. Recently, circRNAs have become a hotspot in scientific field. In this review, we describe the biogenesis, classification, biological characteristics, functions and association with human disease of circRNAs. The emergence of circRNAs will provide a new way to study the pathogenesis of human disease, such as nervous system disease, tumor and ageing.

**Keywords:** circular RNA, biological function, nervous system disease, tumor, ageing

## 1. Introduction

In 1976, Sanger and fellow scientists made the groundbreaking discovery of circular RNA (circRNA) in pathogenic single-stranded cyclic-like viruses responsible for diseases in higher plants [1]. Subsequently, Hsu and Coca-Prados of Rockefeller University identified circRNA in the cytoplasm of the eukaryotic cell line HeLa in 1979 [2]. Further research unveiled the presence of circRNA in diverse biological contexts, such as the cytoplasm of Hepatitis-infected cells [3], yeast mitochondria [4], mouse spermatozoa [5], Drosophila [6], and archaeal bacteria [7-9], establishing the widespread occurrence of circRNAs.

Although circRNAs had been observed in earlier studies, they were initially regarded as seemingly meaningless RNAs or intermediates of lasso structures due to mismatch shearing, and consequently, did not attract much attention. However, recent studies, facilitated by the advancement of high-throughput sequencing and RNA sequencing (RNA-seq) technology, have uncovered a wealth of information about circRNAs, demonstrating their increasingly recognized roles in molecular mechanisms and physiological activities.

The utilization of RNA-seq technology has led to the continuous identification of a substantial number of circRNAs [10]. These studies have revealed that circRNAs are prevalent in archaea, exhibiting evolutionarily conserved sequences and unknown biological functions. For instance, Jeck and colleagues [9] detected over 25,000 circRNA species in human fibroblasts. In our investigation, we conducted a comprehensive search using the circBase database (http://www.circbase.org/cgi-bin/downloads.cgi), yielding a dataset of 92,375 human circRNAs along with their corresponding genomic coordinates. Additionally, analysis of the circRNADisease v2.0 database (http://cgga.org.cn/

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circRNADisease/) uncovered 6,998 circRNA disease entries across multiple species, encompassing 4,246 circRNAs, 330 disease types, and 12 species, including human, mouse, boar, Hidradenitis elegans cryptic nematode, chicken, bovine, goat, porcine, rat, sheep, viral, and Japanese seabream [10].

## 2. Properties of circRNAs

CircRNAs is a class of ring-shaped gene products, single-stranded closed loop, the absence of the 3' end of the hat CAP structure and 5' poly A tail structure, connected by 3'-5' phosphodiester bonds, generally composed of exons and introns, some special regions can be polypeptide coding. The exons are called exonic circRNAs, the introns are called circular intron RNAs, and the exons and introns together are called exon-intron circRNAs. Most circRNAs are predominantly exonic in structure and are mostly localized in the cell cytoplasm. The absence of free 5' and 3' ends prevents ribonuclease R (RNase R) or other nucleases from acting on circRNAs. The structure of circRNAs is more conserved and stable than that of normal linear RNAs, and the half-life of most of them is greater than 48 h. Sometimes, intracellular circRNAs are more stable. The half-life of most circRNAs is greater than 48 h. Sometimes the abundance of intracellular circRNAs is higher than that of linear LncRNAs [11, 12].

There are many types of circRNAs, which are widely present in the biological world in stable forms ranging from hundreds to thousands of lengths, and are found in cells, serum, exosomes, and saliva, and are distributed in various organs in the organism [13].

Most circRNAs have highly conserved sequences, and most of them are evolutionarily conserved, with only a very few being evolutionarily unconserved. It has been shown that the content of many circRNAs expressed does not depend on the expression synthesis of parental linear mRNAs, but rather the expression is regulated at different growth stages. Both the classification and abundance of circRNAs presence can vary greatly in different cellular tissues, with both temporal and spatial specificity. It has been claimed that the diversity of circRNAs species increases through evolutionary upgrading of species, and that the vast majority of circRNAs formation processes are driven by short interspersed nuclear repeats (SINE)[14, 15] .

## 3. Formation of circRNAs and regulatory factors

circRNAs are formed by the shearing action of introns and exons, and can be categorized into three types according to their origin as well as their structure, i.e., exon-originated circRNAs, intron-originated circRNAs, and circRNAs composed of both exons and introns [16]. The current study shows that the three types of cyclic molecules correspond to completely different production mechanisms, with exon-originated circRNAs accounting for the majority of circRNAs, which are widespread in the cytoplasm. About 20% of circRNAs of intron origin may not be stabilized. Current research suggests that circRNAs are reverse sheared into rings from mRNAs [17].

### *3.1. circRNAs of exonic origin*

Jeck [10-11] et al. proposed two models for exon circRNAs: a cyclization model driven by the generation of lasso structures and an intron pairing-driven cyclization model.

### *3.2. Shearosome-mediated formation of circRNAs*

When precursor RNA (pre-RNA) is transcribed, part of the region undergoes folding folding, exon jumping, and as the folding spatially indents the distance between neighboring exons, the site that is spanned forms the lasso structure of the intermediate, and the 3' end of the SD of the downstream exon is attached to the 5' end of the SA of the upstream exon to form the lasso after splicing is performed, and the intronic sequence is removed to form a circular RNA [11-12].

### *3.3. Reverse complementary sequences mediate the formation of circRNAs*

CircRNAs is composed of pre-mRNA reverse shear, pre-mRNA contains long flanks on both sides of the exon, mainly complementary repeat sequences formed by introns, two neighboring introns due to

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base complementary pairing, for binding, and then shear the intron to form a ring. In the study, it was found that since reverse splicing of the RNA itself occurs more often relative to the first exon jumps, reverse splicing of itself is likely to be a normal physiological mechanism. The higher the matching of intronic regions on both sides of the precursor sequence, the more looped RNAs are generated [17-19].

Intronic circular RNA was only discovered in 2013 and is mainly found in the nucleus of human cells, and is considered to be a lasso intron debranching error with tissue specificity. The lasso structure formed by most introns is degraded quickly after debranching in most cases, and if there are some specific sequences of introns that are not broken down by enzymes, intron-derived circRNAs are formed. since they are produced in debranching, the molecules are formed by cyclization of the 2'-5' phospholipid bonds and therefore do not generate the 3'-5' phospholipid bond cyclization typical of circRNAs [23].

### *3.4. Exon-intron circRNAs*

During reverse splicing to form exonic circRNAs, circRNAs molecules with unspliced introns can be stabilized, possibly as intermediates or as a class of independently existing circRNAs molecules. It is mainly present in the nucleus and may regulate parental gene transcription .

Regarding the driving factors of RNA ring presentation, scientists have found that the following points may promote back-to-back shearing patterns and accelerate ring RNA formation:

1. length of exons. The greater the length of the exon sequence, the more likely ring formation.

2. when reverse splicing, the site where the inversion starts can promote the occurrence of cyclization if it is enriched with ALU repeat sequences, e.g. usually greater than 5-fold [10, 20].

3. On both sides of the reverse splicing site, the presence of introns is more likely to form a loop [24].

## 4. Functions of circRNAs

The function of circRNAs is a hot issue in research, and hundreds of thousands of human circRNAs have been identified [24], and one or two regulatory models cannot completely summarize the function of circRNAs. The sequence, structural features and in vivo localization of circRNAs determine the function of circRNAs. The functions of circRNAs with different structures are completely different, so the discussion about the functions of circRNAs must be based on specific molecules, and the known models of circRNAs functions include [22]:

1. sponge (sponge) action: competitive endogenous RNA (ceRNA), competing for binding miRNAs, acting on target genes;

2. binding to proteins and forming complexes to regulate the expression of other molecules;

3. circRNAs directly regulates RNA expression by means of clip complementary pairing;

4. cirRNAs carrying introns promote gene transcription.

The role of circRNAs is mainly can regulate cell signaling pathways and encode polypeptides, of course, there are also circRNAs as miRNA Sponge exist in exosomes to play a role [20, 22].

## 5. Conclusion

The formation mechanism of circRNA has been basically clear, emerging research to explore the discovery of circRNA, such as the process of change about circRNA with the growth and aging of the body, the precise regulatory mechanism of the generation of specific sites, the molecular regulatory pathway of selective access to the exosome, including large, organized, time-specific circular RNA function.

With the development of new generation molecular detection technologies, circRNA has been reported in a large number of literature. Based on the fact that circRNAs are of great significance for the molecular diagnosis of diseases and the assessment of efficacy and prognosis, the biological functions of cyclic RNAs and their roles in diseases need to be further explored and investigated.

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