Molecular Therapy
Nucleic Acids
Review

Recent research progress on circular RNAs: Biogenesis, properties, functions, and therapeutic potential

Ashish Ranjan Sharma,[1,4] Manojit Bhattacharya,[2,4] Swarnav Bhakta,[3] Abinit Saha,[3] Sang-Soo Lee,[1] and Chiranjib Chakraborty[3]

[1]Institute for Skeletal Aging & Orthopedic Surgery, Hallym University-Chuncheon Sacred Heart Hospital, Chuncheon-si 24252, Gangwon-do, Republic of Korea; [2]Department of Zoology, Fakir Mohan University, Vyasa Vihar, Balasore 756020, Odisha, India; [3]Department of Biotechnology, School of Life Science and Biotechnology, Adamas University, Barasat-Barrackpore Road, Jagannathpur, Kolkata, West Bengal 700126, India

Circular RNAs (circRNAs), an emerging family member of RNAs, have gained importance in research due to their new functional roles in cellular physiology and disease progression. circRNAs are usually available in a wide range of cells and have shown tissue-specific expression as well as developmental specific expression. circRNAs are characterized by structural stability, conservation, and high abundance in the cell. In this review, we discuss the different models of biogenesis. The properties of circRNAs such as localization, structure and conserved pattern, stability, and expression specificity are also been illustrated. Furthermore, we discuss the biological functions of circRNAs such as microRNA (miRNA) sponging, cell cycle regulation, cell-to-cell communication, transcription regulation, translational regulation, disease diagnosis, and therapeutic potential. Finally, we discuss the recent research progress and future perspective of circRNAs. This review provides an understanding of potential diagnostic markers and the therapeutic potential of circRNAs, which are emerging daily.

INTRODUCTION

Circular RNAs (circRNAs), an enigmatic and exciting family member of RNAs, have started to gain recognition in recent years.[1,2] Progress in research on circRNAs is moving at a rapid pace (Figure 1). These molecules are single-stranded transcripts that are covalently closed and are generated from precursor mRNA (pre-mRNA) through the backsplicing process, also known as alternative splicing.[3,4] During this non-canonical process, a splice donor site (downstream 5′ splice site) is covalently linked to an upstream region (upstream 3′ splice site).[1] Previously, circRNAs were thought to be a by-product of unusual splicing with no function or minute functional activity, including circRNAs from the *Sry* (sex-determining region Y) gene in adult mouse testes.[4] However, due to rapid progress in the field of high-throughput RNA sequencing (RNA-seq) and circRNA-specific computational biology, a wide array of circRNAs have been reported in eukaryotes, and their functions are being explored.[5–11] The identified circRNAs in eukaryotes have been found in different metazoans, namely, fungi, worms, fish, insects, mammals, and plants.[7,8,11–15] Although circRNA is a single-stranded RNA, it varies from linear RNA for being closed covalently, which imparts circRNA some fascinating properties such as parental gene modulation, protein complex scaffolding, microRNA (miRNA) sponging, and RNA-protein interaction.[2,16] Despite not having polyadenylation (poly(A)) and caps, usually, circRNAs can localize to the cytoplasm and thus this might be the reason for their unique functional abilities.[17] However, through backsplicing from pre-mRNA exons, it is not easy to locate or annotate through an experiment such as polyadenylated RNA-seq. Due to the lack of a 3′ end in circRNAs, these non-coding RNAs are resistant to digestion by ribonuclease R (RNase R). Thus, in a whole RNA pool, circRNAs remain undigested, while other RNAs are digested through RNase R; hence, it helps to easily access these circRNA molecules for next-generation RNA-seq followed by specific computational studies.[18,19]

It is imperative to mention that among several circRNA subtypes, four main subtypes of circRNAs are present. One of them includes exonic circRNAs (ecircRNAs), which are derived from single or several exons, as well as intronic RNAs. Another circRNA is a pre-tRNA intronic circRNA, which is generated by splicing pre-tRNA introns.[20] Exon-intron circRNAs are produced for the retention of the internal intron. The production of circular intronic RNAs (ciRNAs) is generated during canonical splicing, generated due to the failure of the debranching of intronic lariats.[21,22] By interacting with snRNP (small nuclear ribonucleoprotein), ciRNAs and intron-exon circRNAs can perform the transcription of an ancestral gene in the nucleus.

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[4]These authors contributed equally

Correspondence: Sang-Soo Lee, MD, PhD, Institute for Skeletal Aging & Orthopedic Surgery, Hallym University-Chuncheon Sacred, Heart Hospital, Chuncheon-si 24252, Gangwon-do, Republic of Korea.
E-mail: 123sslee@gmail.com
Correspondence: Chiranjib Chakraborty, PhD, Department of Biotechnology, School of Life Science and Biotechnology, Adamas University, Barasat-Barrackpore Road, Jagannathpur, Kolkata, West Bengal 700126, India.
E-mail: drchiranjib@yahoo.com

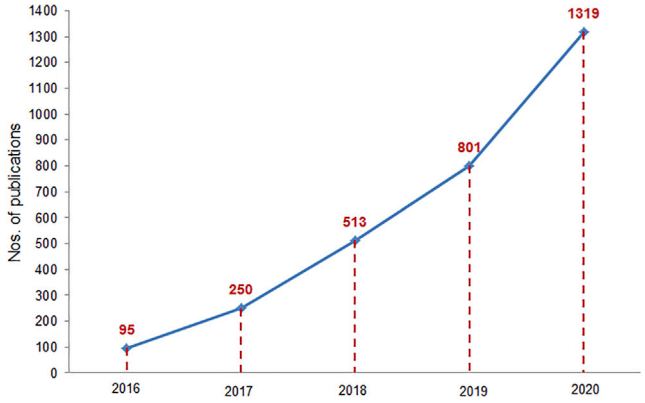

Figure 1. Recent research progress as shown by a circular RNA PubMed search was performed using the term "circular RNA" (2016–2020)

After the discovery of the first circRNA (plant viroid) through an electron microscope, the aspect of backsplicing model circRNA formation was changed thoroughly with the mounting evidence of thousands of circRNAs in several mice and human cell lines.[23,24] After that, several different discoveries of circRNAs created a new line of research (Figure 2). This review highlights a few interesting facts about circRNAs, including their biogenesis and functional characterization. Moreover, the review will help to understand different models of the biogenesis process of circRNAs and types of circRNAs concerning the biogenesis process. The review also discusses different properties of circRNAs such as localization after biogenesis, structure and conserved nature, stability, and expression specificity. Furthermore, a discussion on the biological functions of circRNAs such as miRNA sponging, cell cycle regulation, cell-to-cell communication, transcription regulation, translational regulation, disease diagnosis, and therapeutic potential is also been included. Finally, we discuss the future perspective and conclusions from a future research perspective. This discussion, in turn, will help researchers to develop a new line of scientific endeavor in the field of RNA biology, which is emerging day by day.

BIOGENESIS OF circRNAs

circRNAs are generated mainly from pre-mRNA through a phenomenon called backsplicing of exons.[25,26] Most circRNAs contain complete exons and originate from protein-coding genes. Nevertheless, circRNAs might also consist of non-coding, antisense, intronic, 3′ untranslated region (UTR), 5′ UTR, or intergenic genomic regions.[22,27] The spliceosome mediates backsplicing of pre-mRNA by connecting a downstream splice donor site (5′ splice site) to an upstream acceptor splice site (3′ splice site).[28] The splicing mechanism of circRNA is controlled by *cis*-acting regulatory and *trans*-acting regulatory factors.[29] Canonical splicing sites and regular spliceosomal machinery are essential for the production of circRNAs. Only after transcriptional completion of their host pre-mRNAs has a significant amount of nascent circRNAs been observed.[30] Moreover, when the polyadenylation signal is mutated in circRNA-producing linear genes in manipulatable vectors, circRNA production is eliminated, implying that circularization is usually a post-transcriptional event.[31] These pieces of evidence suggest that circularization might be a post-transcriptional event, but this is not feasible at all times. Recently, a poly(A) signal has been demonstrated not to be required for circRNA production from minigene vectors, suggesting the possibility of the co-transcription process during circRNA formation.[32] Recently, backsplicing of circRNAs from exons has been shown to possibly occur post- or co-transcriptionally.[30] Thus, we may assume that in the case of circRNA biogenesis, both post- and co-transcriptional splicing are involved. However, it may not depend on just 3′ end processing; instead, other factors such as transcriptional elongation rate, availability of spliceosome components, and repetitive elements, among others, might also play a critical role.[30,32,33] Recent pieces of evidence favor the notion that circRNA processing might largely depend on post-transcription rather than co-transcription.[30,31] Metabolic tagging of nascent RNAs with 4-thiouridine (4sU) during circRNA processing revealed that mainly circRNAs are post-transcriptionally processed and are stable.[30] For circRNA biogenesis, intronic repeats and exonic sequences must cooperate, and a functional 3′ end processing signal is vital.[31] Moreover, their formation might be restricted and controlled by *cis*-complementary elements.[30] Thus, circRNA biogenesis appears to depend mostly on post-transcriptional processes rather than co-transcription, but clear insight into this process is still a prerequisite, and in the near future, researchers might further decipher this dilemma.

Types of circRNAs with respect to the biogenesis process

Several types of circRNAs are formed from various biogenesis processes, including exon circRNAs (EcircRNAs), intrinsic circRNAs or ciRNAs, and intron-exon circRNAs (also called exonic-intronic circRNAs (EIciRNAs)) (Figures 3A–3E).[34,35]

EcircRNAs are the most common type of circRNA. Most EcircRNAs are formed from the coding gene of pre-mRNA without involving protein-coding genes (Figure 3A).[9,12] Certain EcircRNAs have the ability to interact with miRNAs and/or RNA-binding proteins (RBPs), and several EcircRNAs interact with other exons containing canonical translation start codons.[2] ciRNA formation depends largely on the conserved sequences at both ends of the introns. ciRNAs contain 2′–5′ phospholipid-linked nucleotides instead of 3′−5′ phospholipid-linked nucleotides (EcircRNAs) and are capable of regulating the expression of genes.[36,37] EIciRNAs, however, contain exons along with introns. As a result, EIciRNAs demonstrate the characteristics and functions of both exonic and intronic circRNAs. Similar to ciRNAs, EIciRNA is abundant in the nucleus.[29] When EIciRNA is bound to RNA polymerase II (Pol II), EIciRNA usually regulates parental gene transcriptional activity by interacting with snRNP.[21]

Different models of the circRNA biogenesis process

Spliceosomes or a group of ribozymes I and II mediates the backsplicing of circRNAs from pre-mRNAs (Figure 3D).[25,38,39] A reduced level of both circRNAs and linear transcripts after inhibition of canonic spliceosomes further confirms the involvement of spliceosomes in the biogenesis of circRNA.[25] Irrespective of orthodox splicing of

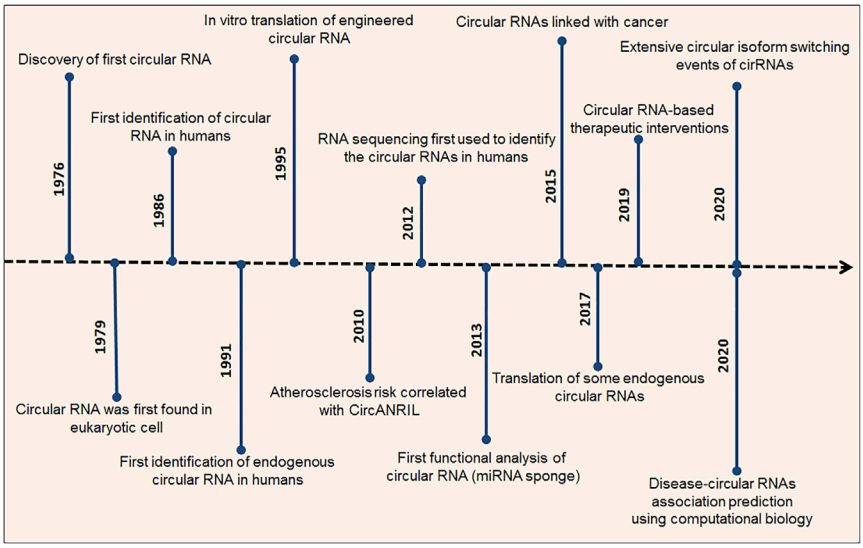

Figure 2. Milestone discoveries in circular RNA research

linear mRNAs, circRNAs can usually arise from a single gene locus. The process usually occurs either through alternative backsplice site selection and/or alternative splice sites. A detail of this process is available in the CIRCpedia database.[40] Certain models of the biogenesis process for circRNAs have been proposed. The most common type of model is exon skipping or the lariat-driven circularization model. In this case, a large lariat comprising the exon(s) is synthesized and successively undergoes internal cleavage to excise the intron and produce ecircRNA or EIciRNA (Figure 3B).[41] In human umbilical vein endothelial cells stimulated by transforming growth factor (TGF)-β or tumor necrosis factor (TNF)-α, RNA-seq datasets showed that most of the skipped exons could produce ecircRNA.[42] The other proposed model is known as the intron pairing-driven circularization or direct backsplicing model. As per this model, exons are split in noncanonical order. Introns containing reciprocal complementary sequences are linked to exons involved in circularization. These introns are spatially close to end-to-end spliced exons that generate circRNAs. However, complementary sequences on either side of an exon do not always produce circRNAs.[43] An *in vitro* minigene constructed model proved that the production of circRNAs does not necessarily require reciprocal complementation between upstream or downstream exon-flanking introns.[28] Various types of models of the circRNA biogenesis process are summarized in Figure 4.

Different aspects of circRNA biogenesis

Recent mutational studies with circRNA expression vectors and HeLa cells treated with the splicing inhibitor (blocks spliceosome assembly) isoginkgetin showed that circRNA biogenesis is mainly dependent on canonical splicing.[25,26]

The efficacy of canonical splicing is much higher than the efficacy of backsplicing. However, the stable mode of circRNA is lower than the stable mode of the mature form of linear RNA. One of the main reasons behind this low efficiency of backsplicing is the unfavorable assembly of spliceosomes, which catalyze the 5′ donor sites of ligation with 3′ acceptor sites of circRNA. However, the exact mechanism involved in backsplicing through the spliceosome is still under investigation. Recently, an analysis of the cryo-electron microscopy structure of the yeast spliceosome E complex revealed that the spliceosome, involving the U1 snRNP and related 3′ splice site (SS) factors (U2AF1, U2AF2, and SF1), can assemble across an exon to define the exon for splicing in a mechanism called exon definition. Splicing through exon definition, rather than the traditional intron definition, leads to backsplicing and circRNA formation.[44] Apart from that, the choice of the circRNA expression pattern, as well as the production pattern of linear RNA and circRNA in the cell, remains elusive. These mechanistic insights may reveal more about circRNA biogenesis.

Although the presence of repetitive elements or RBP interactions can facilitate the looping of the flanking introns to enhance backsplicing, these elements are regarded as dispensable. Studies have demonstrated that backsplicing can occur on substrates with minimal introns (~100 nt) containing only the major splicing signals (5′ SS, 3′ SS, branch site [BS], and polypyrimidine tract [PPT]). Therefore, looping might not be necessary. Nevertheless, the presence of canonical splicing sites has been shown to be indispensable for circRNA formation.[26,44] The looping process is regulated by base pairing between the repeat sequence, such as Alu elements, which are present both in the upstream introns and downstream introns.[39,45] However, looping can also be facilitated through RBP dimerization. In this case, the RBPs may bind to particular motifs within the flanking introns.

Thus, in addition to *cis*-elements, RBPs such as FUS (fused in sarcoma) and QKI (quaking; encoded by the *QKI* gene) can regulate the biogenesis of circRNA (Figure 3C).[46,47] FUS has been shown to regulate the production of a considerable number of circRNAs in *in vitro*-derived mouse motor neurons (MNs). FUS accomplishes this regulation by binding the introns flanking the backsplicing junctions during circRNA biogenesis.[46] To enhance the production of numerous circRNAs, QKI binds to recognition elements. During the epithelial-to-mesenchymal transition (EMT), QKI was observed to be involved in the upregulation of circRNAs in humans.[47] QKI, which itself is regulated during EMT, is responsible for producing more than one-third of abundant circRNAs. QKI motifs, when added, are enough to induce *de novo* circRNA synthesis from linearly spliced transcripts. Hence, the insertion process of synthetically QKI-binding sites related to introns may be adequate for the production of circRNAs. The study concluded that cell type-specific mechanisms could purposefully synthesize and regulate circRNAs and

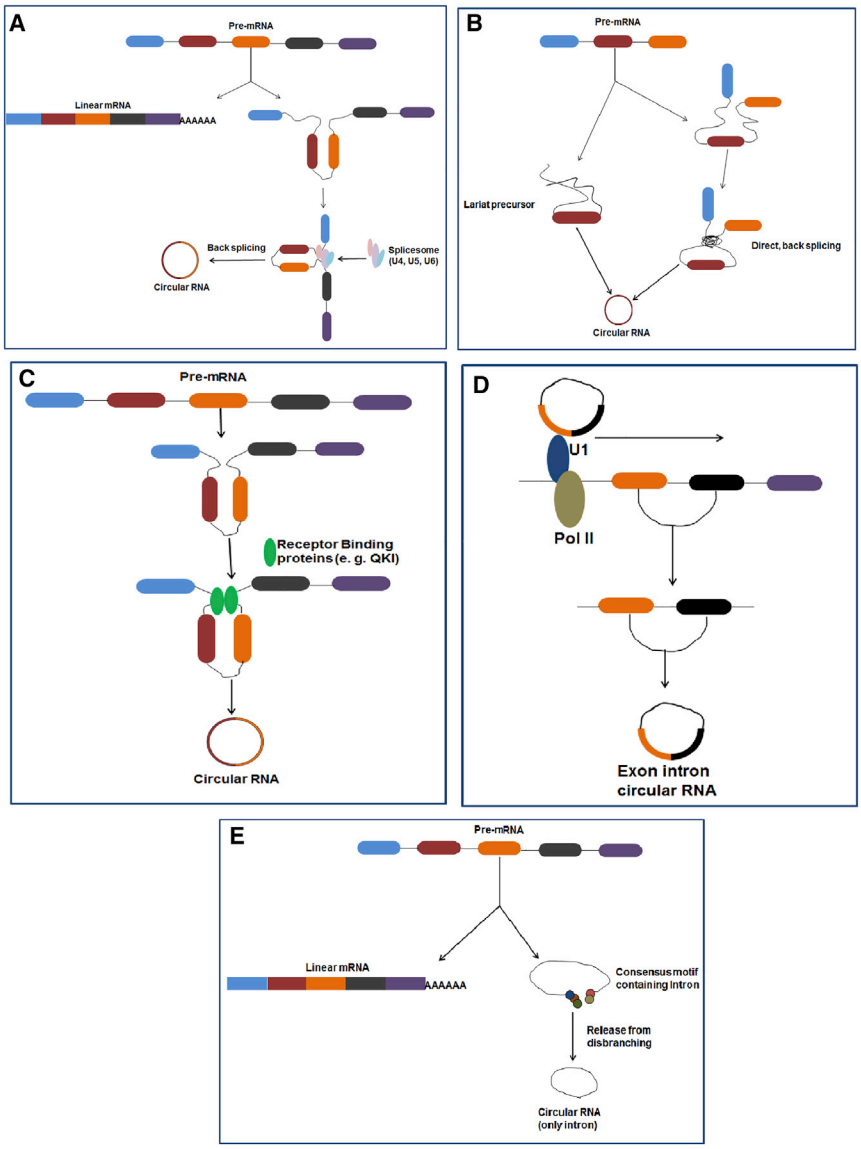

Figure 3. Different proposed biogenesis mechanisms of circular RNA

(A) Spliceosome-dependent biogenesis of circular RNA. (B) Circular RNA biogenesis occurs through direct back-splicing from pre-mRNA. (C) Circular RNA biogenesis occurs by using RNA-binding proteins (RBPs). (D) Exon-intron circRNA (ElciRNA) biogenesis produced by back-splicing. ElciRNAs contain both exons and introns. (E) Circular intronic RNA (ciRNA) biogenesis process. This ciRNA was derived from lariat introns during canonical splicing.

play explicit biological functions during EMT.[47] The specific adenosine deaminase enzyme related to double-stranded RNA (dsRNA) inhibits base pairing of repetitive elements flanking introns, which in turn inhibits looping and affects the biogenesis of circRNAs and expression. That specific adenosine deaminase enzyme related to dsRNA is coupled with the editing of adenosine to inosine in endogenous dsRNA[48] and an enzyme called ATP-dependent RNA helicase A.[49] This complex prevents the activation of the innate immune system. Thus, the looping of intron sequences is prevented by this unwinding of dsRNA helical configuration as well as the editing of adenosine to inosine.[8,50]

Additionally, the biogenesis and expression of circRNAs are regulated by epigenetic regulation of histone proteins and genetic material. Recent studies have shown that silencing the *DNMT3B* gene, which produces DNA methyltransferase, can affect circRNA expression.[51–54] Thus, the role of gene methylation in the biogenesis and expression of circRNAs indicates circRNA formation. However, the epigenetic regulation status of the promoter region of the host gene can be influenced by circRNAs. The friend leukemia integration 1 transcription factor (*FLI1*) gene-encoded protein in breast cancer cells is one of the crucial examples of this event.[55]

DIFFERENT PROPERTIES OF circRNAs

Localization after biogenesis

Except for intron-containing circRNAs, most circRNAs are generally exported from the nucleus to the cytoplasm just after their biogenesis.[1,9,56] Intronic circRNAs and EIciRNAs have been noted to be extensively found in the nucleus.[52,53] This transportation depends on the length of circRNAs and is facilitated by UAP56, a spliceosome RNA helicase, and URH49, an ATP-dependent RNA helicase (Figure 5).[54] The export phenomena of circRNAs vary from species to species based on their different length requirements for exporting circRNAs. One example is the export of short circRNAs (<400 nt) related to URH49 in humans. Likewise, the exports of long circRNAs (>1,200 nt) are related to U2AF65-associated protein 56 (UAP56). The conserved K-K/SL-N motif of UAP56 is associated with the export phenomenon of long circRNA through the nuclear pore to the cytoplasm. At the same time, the R-S-F-S motif of URH49 is responsible for the export process of short circRNAs. Interestingly, when URH49 is mutated to contain the Hel25E/UAP56 motif and vice versa, the K-K/SL-N motif for UAP56 and the R-S-F-S motif of URH49 are observed to be sufficient and necessary to dictate circRNA length preferences. This phenomenon subsequently results from the change of behavior of URH49 to Hel25E/UAP56 and vice versa due to the presence of swapped motifs.[57] How 7-methylguanosine (m^7G)-capped RNAs are exported in cells can explain the role of RNA length in controlling the nuclear export of circRNAs. Capped small nuclear RNAs (snRNAs) (<200 nt) are exported via the PHAX (phosphorylated adaptor

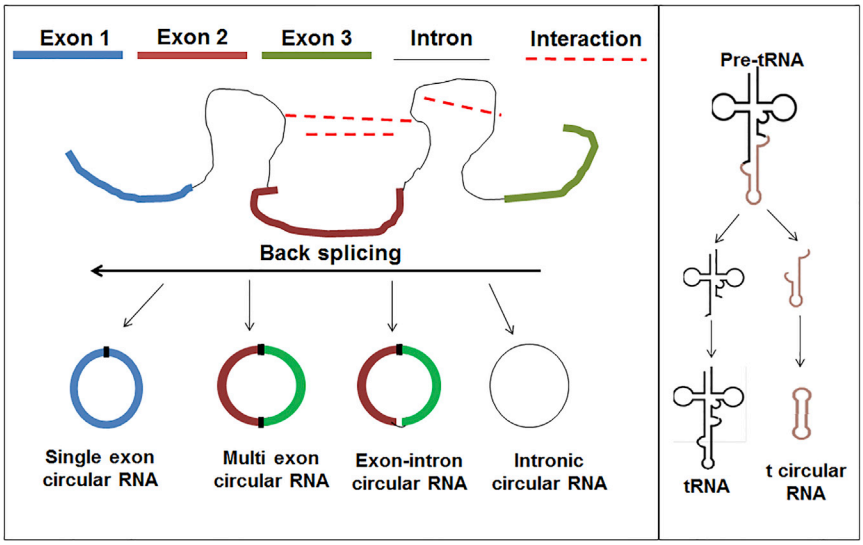

Figure 4. Different types of circular RNA regarding the biogenesis model

RNA export protein)/CRM1 (chromosome region maintenance 1)-mediated pathway but become exportable via NXF1 (nuclear RNA export factor 1)-NXT1 (nuclear transport factor 2-like export factor 1) when their length is increased. Similarly, long capped mRNAs are exported via NXF1-NXT1, but when their length is shortened (intron-less mRNAs), they are exported via the PHAX/CRM1-mediated pathway.[58] Localization of circRNAs thus depends on their types (Table 1). Due to the variable lengths, sequences, and structures of circRNAs, they can be fed into specific nuclear export pathways, which further depend on the evolutionarily conserved factors responsible for measuring the length of the mature circRNAs. However, this hypothesis needs further experimental validation.[57]

Structure and conserved nature of circRNAs

PSTVd (potato spindle tuber viroid) was the first discovered circRNA and contained rod-shaped, monomeric structures with several distinct loops.[59] Liu et al.[60] demonstrated 26 circRNA structures (16–26 bp long) from diverse cell lines, except circDHX34 and circPPP1CB. circDHX34 is 29 bp long, and circPPP1CB is 32 bp long. Researchers have shown that endogenous circRNAs can form imperfect RNA duplexes to inhibit dsRNA-activated protein kinase R (PKR). In an autoimmune disease such as systemic lupus erythematosus (SLE) or viral infection, circRNAs undergo massive and rapid degradation by RNase L. This process is essential for PKR activation in the early stage of the innate immune response.

Secondary structures similar to circRNAs may recognize endoribonuclease dicer proteins for the biogenesis of miRNAs. However, the probable molecular mechanism of miRNA production from circRNAs has yet to be discovered. Recent studies have shown that lengthy circRNAs from a plant species (*Oryza sativa* ssp. *indica*) include multiple miRNA sequences.[61] Apart from that, internal modification may also facilitate mRNA processing. The most abundant internal modification of mRNAs is *N*6-methyladenosine (m^6A). This internal modification has been implicated in all aspects of post-transcriptional RNA metabolism associated with mRNA and long non-coding RNAs (lncRNAs).[62] Recently, m^6A modifications have also been found in circRNAs. m^6A circRNAs mediate mRNA stability via YTHDF2 proteins. Nevertheless, degradation of circRNAs is not promoted by m^6A circRNAs as it does for mRNAs.[63]

Recent studies informed the conservation of circRNA using RNA sequences. The studies include genomic loci and inform the orthologous and paralogous nature of evolution. The conserved nature of circRNAs has been depicted in several species of plants and animals. The conserved nature of circRNAs originates from different genes such as *PVT1*, *HIPK2*, *IPW*, and *KIAA0182*.[9,10] Similarly, the conserved nature of circMBNL1 was expressed in *Drosophila* heads and humans.[24] In addition to animals, approximately 700 conserved ecircRNAs were noted in both plant species (*A. thaliana* and *O. sativa*).[64] A screening of circRNAs from humans, macaques, and mice also revealed the evolutionarily conserved nature of circRNAs that could be used for prioritization and functional screening. The study tried to utilize the conserved nature and co-expression networks to prioritize circRNAs for the involvement in liver tumerogenisis.[65] Another evolutionarily conserved circRNA, GW182, from *Drosophila* to humans, is shown to have a role in the degradation of other circRNAs.[66]

Stability and expression specificity

Due to the unique covalent bond between the 5′ and 3′ ends, circRNAs are resistant to RNA exonuclease degradation. Moreover, the absence of a 2′–5′ linkage of an RNA lariat makes the circRNAs resistant to RNA debranching enzymes. circRNAs have long half-lives because of these characteristics. circRNAs have been shown to be stable in cells and exhibit a half-life of more than 48 h compared to mRNAs (10 h).[3,9] However, due to the presence of circulating RNA endonucleases in serum, stability for circRNAs is only <15 s.[67]

The expression profile of circRNAs depends on several factors such as tissue, type of isoforms, and developmental stages, reported in several cases such as WI-38 fibroblast cells, *O. sativa,* and *A. thaliana.*[1,10,64,68–70] During drought stress in *Triticum aestivum*,[71] cold tolerance in *Vitis vinifera*,[72] and phosphate imbalance in *O. sativa*,[64] the expression of some stress-specific circRNAs has been reported. In several developmental stages of a plant (*Phyllostachys edulis*), the differential expression profile of circbHLH93 has been documented.[73] One of the most reported tissue/cell-specific circRNAs is circSRY, which shows its expression in adult mouse testes. However, linear Sry mRNA is present only in the tissue of the developing genital

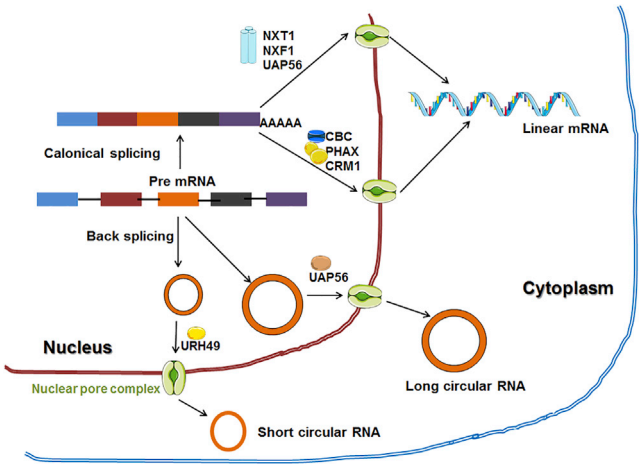

Figure 5. Export mechanism of the circular RNA from the nucleus to the cytoplasm

The export mechanism has occurred through the length-dependent mode.

ridge.[4,74] Another example is the *DCC* gene, where expression of the circular isoform varies among various human tissues, but its cognate linear mRNA expression is not correlated. In some instances, despite high levels of mRNA expression, no circRNA was detected.[23]

Meanwhile, it is also true that the expression of some circRNAs is more abundant than the expression of their linear counterparts. In several cell lines, the expression of circCAMSAP1 is several-fold higher than the expression of its linear counterpart. A similar kind of overexpression of circRNA of approximately 50 different genes was observed in different cell lines, i.e., HeLa, AG04450, and A549 cell lines.[10] Similarly, in *O. sativa* ssp. *indica*, approximately 20% of circRNAs were found to be expressed at higher levels than in their linear counterparts.[9]

FUNCTION

The functional mechanism of circRNAs includes mainly acting as miRNA sponges, regulating gene splicing or transcription, translation, interacting with proteins, and epigenetic regulation. Usually, due to genetic code redundancy, the third position is not highly conserved in a codon. However, in some circRNAs, the third position is more conserved than in exons (not part of circRNAs), suggesting that these highly stable circRNAs have significant non-coding functions. circRNAs can act as post-transcriptional regulators by competing with other RNAs (for binding with miRNAs and RBPs).[1] If functional classification is broadly performed according to the target of circRNAs, then they can generally be divided into two categories: regulating the target site and regulating its host gene. However, a recent investigation suggested that circRNAs show different physiological functions and regulate gene expression at various levels.[75]

miRNA sponges: A novel function of circRNAs

miRNA sponging is one of the well-established functions of circRNAs, where they can inhibit the function of miRNA by binding the target miRNAs directly or indirectly (Table 2). The relationship between the circRNA interaction site and miRNA as well as the mRNA target site is very significant for this functional consideration. circRNAs possess miRNA response elements as linear RNAs, allowing them to compete with miRNA binding to linear RNAs, suggesting a regulatory role in miRNA functioning and gene expression.[9,93] Moreover, this study indicated that RNA interference (RNAi) may target circRNAs. Therefore, to support this model, one study was carried out on ciRS-7, which is a special type of circRNA sponge for miR-7. The ciRS-7 was produced from a *CDR1* antisense transcript with approximately 74 miR-7 binding sites in humans as well as approximately 63 conserved binding sites from different vertebrates (32 vertebrates). In particular, ciRS-7 has highly stable expression in several tissues, specifically in the brain.[1,94,95] The capacity of ciRS-7 to bind almost 20,000 miR-7s in each cell is due to its high expression potential. ciRS-7 can influence the binding capacity of miR-7 with target mRNAs, which are involved in various cancers[95,96] and neurodegenerative disorders such as Alzheimer's disease[77] and Parkinson's disease.[97] Hence, if the expression of ciRS-7 can be controlled, then it can regulate mRNA expression along with the binding sites of miR-7. This study further suggests that there is competition between ciRS-7 and mRNAs for miR-7 binding.[2] Apart from that, ciRS-7 has a complementary binding site with miR-671, which causes the cleavage of ciRS-7 by Argonaute-2 (AGO-2).[98] In addition to ciRS-7, another circRNA (testis-specific circRNA), *circSRY*, is an example that has several target sites for miR-138 (16 target sites in mice) and might play a crucial role in the progression of several diseases (cancer and Parkinson's disease).[2,99]

A single circRNA can bind to not only a single miRNA but also many miRNAs at one or more sites. One example is circFOXO3, which can bind with different miRNAs such as miR-138, miR-22, miR-762, miR-136, miR-433, miR-3622b-5p, miR-149, and miR-3614-5p. Another example is circITCH, which can bind to miR-124, miR-17, and miR-138.[61,99] However, from 16 different plants, 115,171 circRNAs were observed. This work demonstrated that the circRNA sponging function is not only observed in humans but is also documented in different plants. However, circRNA sponging is observed more often in different diseases.

Cell cycle regulation and cell-to-cell communication

Numerous circRNAs show different mechanisms of action due to various binding sites to miRNAs as well as proteins related to cell cycle regulation. For example, upon binding to miR-762, miR-138, miR-433, miR-22, miR-149, miR-3622b-5p, miR-96, miR-136, and miR-3614-5p, circFOXO3 sponges the binding of these miRNAs to the linear variant of FOXO3. This binding relives its suppression.[100,101] Similarly, the binding sites of circFOXO3 control cell cycle regulatory proteins such as p53, p27, p21, mouse double minute 2 (MDM2), and cyclin-dependent kinase (CDK)-2. Using pull-down assays, the role of circRNA-protein interactions was studied for cell cycle regulation.[102,103] Studies by Du et al.[102] demonstrated that CDK-2 and p21 bind to adjacent sites of circFOXO3 to form a ternary complex. This complex arrests the cell cycle in the G_1 phase by

Table 1. Different circRNAs: localization, mode of action, and biological functions

circRNA	Mode of action	Biological functions	References
Cytoplasm			
ciRS-7 (also called CDR1as)	miRNA sponge for miR-7	positive regulator of insulin secretion, neuronal development, oncogenic functions	1,2,76,131,170–172
circHIPK3	miRNA sponge for multiple miRNAs	tumor suppressor, positive regulator of insulin secretion	132,172,173
circZNF91	miRNA sponge or decoy for miR-23b-3p	differentiation of epidermal stem cells	51
circBIRC6	miRNA sponge or decoy for miR-34a and miR-145	pluripotency maintenance	174
circMbl	protein sponge or decoy for Mbl and template for translation	regulator of neuronal functions	26,27
circCCDC66	miRNA sponge or decoy for multiple tumor suppressor miRNAs	oncogenic functions	175
circPVT1	miRNA sponge or decoy for miR-497-5p	positive regulator of cell cycle progression	176
circANRIL	protein sponge or decoy for PES1	impairs pre-rRNA processing and ribosome biogenesis to induce nucleolar stress and activate p53	177
circPABPN1	protein sponge or decoy for HUR	suppresses PABPN1 translation and decreases cellular proliferation	178
circ- Amotl1	protein scaffold (facilitates PDK1-dependent phosphorylation of AKT1)	cardioprotective role in doxorubicin-induced cardiomyopathy	179
circ-Foxo3	protein scaffold (facilitates MDM2-dependent ubiquitylation of p53) and sponge for MDM2 (to prevent ubiquitylation of FOXO3)	induces the apoptosis of cancer cells	103
circ-ZNF609	template for translation	regulates myoblast proliferation	114
circPINTexon2	template for translation	tumor suppressor	180
circ-FBXW7	template for translation	tumor suppressor	181
circ-SHPRH	template for translation	tumor suppressor	182
Nucleus			
circPAIP2	enhances protein function (positive regulator of RNA Pol II transcription)	positively regulates the expression of its parental gene	21
circEIF3J	enhances protein function (positive regulator of RNA Pol II transcription)	positively regulates the expression of its parental gene	21
ci- ankrd52	enhances protein function (positive regulator of RNA Pol II transcription)	positively regulates the expression of its parental gene	22
FECR1	protein recruitment (recruits TET1 to the promoter region of its own host gene)	oncogenic functions through upregulation of FLI1	55
cia-cGAS	protein sponge or decoy for cGAS	protects long-term hematopoietic stem cells from exhaustion	133

FECR1, FLI1 exonic circular RNA; MDM2, mouse double minute 2; PABPN1, poly(A) binding protein 1; PDK1, phosphoinositide-dependent protein kinase 1; PES1, pescadillo homolog 1; TET1, tet methylcytosine dioxygenase 1.

inhibiting the activation of the complex (cyclin E/CDK-2), which is required for the G_1/S transition.

In recent studies, overexpression of circRASSF2 was identified in circulating exosomes from patients suffering from laryngeal squamous cell carcinoma (LSCC).[104] Within the different lengths of circulating exosomal circRNAs, tiny-sized circRNAs, encircled by exosomes circulating in the blood of animals, can act as significant biomarkers as well as cell-signaling molecules. Additionally, changes in the miRNA levels in producer cells may regulate the sorting of associated circRNAs to exosomes. This, in turn, might result in the transmission of the biological activity to recipient cells.[105]

Transcription regulation

circRNAs are able to regulate transcription and alternate splicing. Recent studies have shown that circRNAs can regulate transcription in both *cis* and *trans* manners. Most circRNAs are located in the cytoplasm,[6,9] and intron-containing circRNAs are found in the nucleus of cells in humans. Linear RNAs with retained introns are passaged into the cytoplasm, and those with detained introns stay in the nucleus.[21,22,106] For example, circPAIP2, circMCM5, circEIF3J,

Table 2. Diseases regulation of circular RNAs through miRNA sponging

SI no.	Circular RNAs	Sponged miRNA	Target gene for miRNA	Different diseases regulation	References
1	CDR1as/ciRS-7	miR-7	*Pax6 and Myrip*	diabetes	[76]
2	CDR1as/ciRS-7	miR-7	*UBE2A*	Alzheimer's disease	[77]
3	circRNA-CER	miR-136	*MMP13*	osteoarthritis	[78]
4	circTCF25	miR-103a-3p and miR-107	*CDK6*	bladder cancer	[79]
5	circRNA-MYLK	miR-29a	*VEGFA*	bladder cancer	[80]
6	hsa_circ_000984	miR-106b	*CDK6*	colorectal cancer	[81]
7	hsa_circ_0020397	miR-138	*TERT* and *PD-L1*	colorectal cancer	[82]
8	ciRS-7	miR-7	*YY1*	colorectal cancer	[22,83]
9	ciR-SRY	miR-138	*TWIST2*	colorectal cancer	[84]
10	ciR-SRY	miR-138	*PCNA; Bcl-2*	ovarian cancer	[85,86]
11	ciRS-7	miR-7	*IGF1R*	gastric cancer	[87]
12	CDR1as/ciRS-7	miR-7	*PI3K*	gastric cancer	[88]
13	circRNA_LARP4	miR-424	*LATS1*	gastric cancer	[89]
14	ciR-SRY	miR-138	*H2AX*	lung cancer	[90,91]
15	ciR-SRY	miR-138	*APT1/2*	chronic lymphocytic leukemia	[92]

APT1/2, acyl protein thioesterase 1/2; Bcl-2, B cell lymphoma-2; CDK6, cyclin-dependent kinase 6; H2AX, histone family 2A variant, member X; IGF1R, insulin-like growth factor 1 receptor; LATS1, large tumor suppressor kinase 1; MMP13, matrix metalloproteinase 13; Myrip, myosin VIIA and Rab interacting protein; Pax6, paired box 6; PCNA, proliferating cell nuclear antigen; PD-L1, programmed death-ligand 1; PI3K, phosphoinositide 3-kinase; TERT, telomerase reverse transcriptase; TWIST2, twist basic helix-loop-helix transcription factor 2 gene; UBE2A, ubiquitin protein ligase A; VEGFA, vascular endothelial growth factor A; YY1, Yin Yang 1.

circANKRD52, and circSIRT7 are introns containing circRNAs that may interact with the elongated RNA Pol II complex to regulate the expression of their genes through a positive feedback mechanism.[22]

Alternative splicing by an intron retention (IR) mechanism can form different isoforms from a single gene. Usually, fully spliced introns in mature mRNA isoforms are passaged out to the nucleus for translation. The presence of premature termination codons (PTCs) in introns causes rapid degradation of intron-retaining isoforms (IRIs) (including circRNAs) mediated by the nonsense-mediated decay (NMD) pathway.[107] Sometimes IRIs can avoid the NMD pathway[108] and get translated to protein isoforms but are often truncated[109] and are toxic to cells. Thus, retained introns after splicing may lead to gene regulation by introducing a stop codon in an ORF and forcing premature termination of translation without affecting the transcriptional activity. At the same time, IRs that are not exported to the cytoplasm but remain in the nucleus are known as detained introns (DIs). A study has shown that CLK1-DIs can serve as a reservoir for the CLK1 mRNA. CLK1 is a dual-specificity kinase responsible for phosphorylating the spliceosomal complex regulating RNA splicing. The study concluded that polyadenylated RNAs having DIs could serve as a reservoir of pre-mRNAs or lead to a dead end by degrading them in the nucleus.[110]

EiciRNAs can express their genes after binding to the U1 spliceosome complex, along with post-transcriptional regulation.[111] However, some circRNAs (e.g., circMBL, circFMN, and circDMD) can suppress expression by binding directly to their cognate mRNAs (mRNA traps).[112] Due to incomplete splicing, some EIciRNAs retain introns that allow them to interact with U1 snRNP and promote their host gene transcription.[21] In *A. thaliana*, exon 6 of a key developmental regulatory gene, SEPALLATA3 (*SEP3*), generates circRNA, of which ~15% of the circular transcript is retained in the nucleus. This circRNA forms an R-loop (an RNA:DNA hybrid) at endogenous *SEP3* genomic loci. The formation of this R-loop results in elevated linear *SEP3* splice variant production with exon 6 skipped. R-loop formation has been suggested to result in a pause of *SEP3* transcription, allowing recruitment of alternative splicing regulators to promote exon skipping.[113] Similar kinds of transcriptional regulation might be expected in mammalian cells, and further research might decipher this process. Thus, circRNAs can play a pivotal role in diverse transcriptional regulations.

Translation of circRNAs

As circRNAs are classified as non-coding RNAs, their role in translation has never caught attention. Several studies have reported the protein-coding function of circRNAs (endogenous) due to polysomal association.[27,114,115] Owing to the absence of the 5′ cap and the poly(A) tail, circRNAs are unable to undergo cap-dependent translation. However, due to the presence of internal ribosome entry sites (IRESs) and the ability to bind to open reading frames (ORFs), circRNAs have been shown to code for proteins/polypeptides.[116] One of the examples is circZNF609, which is composed of two in-frame start codons that are separated by 150 nt and are found in myoblast cells. This circRNA has a 5′ IRES (conserved IRES). Thus, cap-independent translation can produce two similar intense proteins, which shows the functional ability of circRNAs in translation. However, certain evidence signifies its role in myogenesis, which helps in the propagation of mouse and

human myoblast cell lines and can be suppressed through the silencing of this circRNA (circZNF609) using small interfering RNA (siRNA).[114] Methylation of the IRES of circZNF609 might be a possible reason for cap-independent translation, which has been investigated through an RNA-wide analysis of the m^6A pattern.[117] A single m^6A site has been observed to be sufficient rather than two to induce circRNA translation with the same efficiency in IRES.[115]

As listed in circRNADb, protein expression was recorded from 72 circRNAs in humans.[118] Apart from this, 250 circRNAs were found to have translatable coding potential, and they can be linked with polysomes.[119] circMBL3 encodes a protein (~37 kDa) that is an endogenous circRNA found in *Drosophila*. From the functional catalog of circRNAs in protein translation, circRNAs have been reported to have a possible role in cancer progression.[120] For example, a product of the *circPPP1R12A* (hsa_circ_0000423) gene (*circPPP1R12A-73aa*, having a 216-nt short ORF) is implicated in cancer progression. Protein from *circPPP1R12A-73aa* has a unique conserved peptide at the C terminus. In nude mice and from 20 different patient samples, circPPP1R12A-73aa protein was shown to be able to regulate colon cancer progression invasion and metastasis. Several colon cancer cell lines (e.g., SW620, HT-116, LoVo, DLD-1, HCT-15, HT-29, SW480, SW48, and Caco-2) also showed expression of circPPP1R12A-73 aa protein compared to the normal cell line (NCM460).[119]

The functional role of most circRNA-derived peptides has yet to be elucidated. However, peptides from circRNAs have been proposed to often be truncated versions of canonical proteins and lack vital functional sites. Therefore, they might function either as dominant-negative protein variants or decoys or modulators of alternative protein complexes.[114] Most circRNAs are synthesized from protein-coding genes. Thus, ORFs in circRNAs often have similarities to the ORFs of the corresponding mRNAs. Hence, the best possible way to detect whether a protein is synthesized from the linear or circular transcript is to characterize the sequence downstream of the back splice junction (BSJ) and before the putative stop codon.[32] A detailed methodology for studying the translation of circRNAs has been reviewed by Kristensen et al.[32] Authors have suggested using several molecular techniques, such as luciferase reporters or analysis of m^6A methylation, to identify the presence of ORF functional IRES-like elements. Mass spectrometry has been applied for detecting circRNA-specific peptides spanning the BSJ and inserting a tag protein in the circRNA ORF (upstream of the putative stop codon) using a minigene setup or CRISPR-Cas9.[121] This tag can be detected by western blotting by using an antibody against the circRNA-specific part of the peptide if available. The tag can also help to study localization by using peptide-specific immunofluorescence. Finally, polysome profiling or ribosome footprinting can be used to study the translated products of circRNAs.

circRNAs and disease diagnosis

For disease diagnosis, next-generation biomarker discovery is an urgent need. In recent years, due to the tissue-specific expression, longevity, and high abundance of circRNAs, many studies have characterized circRNAs, which highlights their potential as biomarkers for certain diseases. Previously, miRNAs and several RNA transcript regions, which can act as stable biomarkers, were explored. Recently, after the discovery of circRNAs, scientists have attempted to establish this molecule as a suitable biomarker through RNA profiling and have studied its sensitivity.[120] The capability of circRNAs to act at the transcriptional and post-transcriptional levels makes them ideal candidates for biomarkers for various diseases such as cardiovascular diseases, neurological disorders, and cancer. circRNAs tend to be released from cells to the circulatory system, which has the potential to serve as a biomarker. circRNAs found in exosomes can be used as potential biomarkers for cancer diagnosis.[105] In colorectal cancer (CRC) patients, exo-circRNAs within serum exosomes constitute a novel and stable class of circRNAs.[105] A correlation between the expression of circ_05075 (hsa_circ_0005075) and tumor size in hepatocellular carcinoma (HCC) suggests that it is a potential HCC biomarker. Although a correlation has been identified between HCC and circRNAs, further research is required to understand the role of circRNAs in HCC development.[122] Stable small-sized circRNAs are found in the circulatory system. circRNAs have also been found to have the potential to cross the blood-brain barrier (BBB), and the circRNAs can be detected upon entering the cerebrospinal fluid (CSF). Thus, these circRNAs indicate central nervous system (CNS) disorders and can act as potential biomarkers.[123]

circRNAs and therapeutic potential

circRNAs are being extensively studied in human diseases. Several circRNAs are differentially expressed in different diseases (Figure 6). Among these circRNAs, some were upregulated, and some were downregulated. Due to their ability to regulate gene expression, circRNAs have come into sight as potential therapeutic molecules. Scientists are targeting different circRNAs that have therapeutic potential. Several circRNAs are being used as therapeutic targets in different diseases. Furthermore, tumor-suppressive circRNAs have been observed to have the potential to act as therapeutic molecules in cancer.[124] However, to understand the therapeutic potential of circRNAs, it is necessary to perform functional circRNA knockdown experiments and/or circRNA overexpression experiments. Using self-splicing introns[125–127] or splint ligation approaches,[39] circRNAs can be generated and added to cells for functional analysis. Overexpression of circRNA can be performed through circRNA overexpression plasmids with the circRNA sequence.[74]

Utilizing endogenous spliceosome machinery, a number of plasmid- and virus-based methods are available for expressing circRNAs of interest. Even in some cases, the production of linear RNAs is minimal.[128–130] Expression of circRNA genes can be achieved using a minigene setup whereby canonical splice sites and inverted complementary sequences flank a circularizing exon[26,31] or the entire gene can be expressed.[74] Naturally occurring repeat elements such as Alu can act as complementary sequences, or these elements can be produced by inserting a region (>30 nt) of the upstream intron downstream of the circularizing exon in an inverted position. Inserting

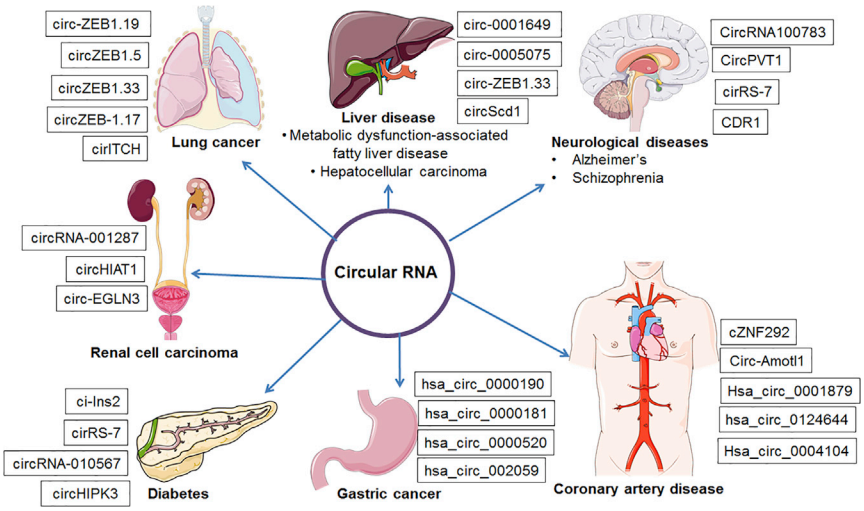

Figure 6. Different circular RNAs associated with different diseases

the binding domains of certain RBPs in the flanking introns (promoting circularization) can further enhance the expression of circRNA transcripts from plasmids.[47] Alternatively, the knockdown of specific circRNAs can be attained using short hairpin RNAs (shRNAs)/siRNAs. These molecules can be used to target the backsplicing junction. CRISPR-Cas9 genome editing can be used to remove flanking repeat elements (or the entire locus in the case of CDR1as/ciRS-7)[131–133]. Recently, for the first time, circRNA knockout animals were generated. Knockout of *CDR1as/ciRS-7* caused impaired sensorimotor gating in mice, a phenotype associated with neuropsychiatric disorders.[131] At the same time, cia-cyclic guanosine monophosphate-AMP synthase (*cGAS*) knockout in mice caused the number of long-term hematopoietic stem cells (associated with severe anemia and death) to be reduced.[133] However, the most successful model will be when phenotypes observed after the knockout of a circRNA can be compensated by a plasmid or viral vector reexpressing the circRNA. For instance, overexpression of a mouse *Fndc3b* gene circRNA via adeno-associated virus (AAV) resulted in better cardiac repair in a myocardial infarction model.[130] Due to the presence of its genome, mitochondria play crucial roles in immuno-metabolic syndromes. Steatohepatitis-associated circRNA ATP5B regulator (SCAR), located in mitochondria, was observed to inhibit mitochondrial reactive oxygen species output and activation of fibroblasts from patients with nonalcoholic steatohepatitis (NASH). *In vivo* study revealed that targeting of circRNA SCAR alleviates insulin resistance and high-fat diet-induced cirrhosis.[134]

New delivery strategies of therapeutic-based circRNAs may further help to explore the therapeutic potential of circRNAs. The different circRNAs with therapeutic potential for different diseases are listed in Table 3.

RECENT RESEARCH PROGRESS ON circRNAs

Several breakthroughs in research that have been performed on circRNAs are illustrated in the following sections.

Recent basic research progress on circRNAs

Although many eukaryotic genes generate linear mRNAs and circRNAs, the extent to which the ratio of linear RNA to circRNA is generated and how it is modulated remain unknown. In *Drosophila* cells, utilizing RNAi screening, many transcription termination factors and core spliceosomes that regulate the RNA outputs of the reporter and endogenous genes have been identified. The steady-state output of protein-coding genes was observed to be diverted toward the synthesis of circRNAs when canonical pre-mRNA processing is slowed down or inhibited. circRNA production is increased due to the activation of alternate pathways (backsplicing), leading to the formation of circRNAs from nascent RNAs. This methodology may provide an alternative pathway for circRNA production.[154] Wu et al.[155] generated a full-length assembly tool, namely, circAST. Depending on the internal structural characteristics of circRNAs, this tool can help analyze the expression of circRNA by using multiple splice graphs and assemble the transcripts of alternatively spliced circRNA. A limitation of the short half-life makes mRNA use in biological systems infeasible. Recently, to extend the duration of protein extension from linear RNA, exogenous circRNA was synthesized. This circRNA was developed by utilizing engineered self-splicing introns. Engineered exogenous circRNAs can produce robust, stable, and potent protein expression in eukaryotic cells and might offer an alternative to linear RNAs.[126] Dong et al.[156] developed a database entitled CIRCpediav2 for circRNA annotation as well as their expression assessment. For annotation, the database used nearly 180 RNA-seq datasets from six different species. Moreover, this web interface provides computational tools for the comparison of the expression of circRNAs. In the developing human frontal cortex, circSLC45A4 is one of the most highly expressed RNA splice isoforms. Knockdown of circSLC45A4 pushes the human neuroblastoma cell line to undergo spontaneous neuronal differentiation. Thus, to maintain the progenitor state of neural cells in the mammalian group brain, circSLC45A4 is necessary.[157] Recent progress in the field of circRNAs would further pave the way for the development of new strategies that could be utilized to address the molecular pathogenesis of several diseases.

For identifying circRNAs, short-read RNA-seq is the most commonly used technique. However, it suffers from the limitation that experimentally it is unable to determine full-length sequences and exact exonic compositions of circRNAs. Due to this, the function of very few circRNAs has been characterized. Knowledge of full-length circRNA sequences could substantially benefit the functional studies of circRNAs.[158] Thus, strategies for reading long-read RNA-seq are being developed. IsoCirc is one of those strategies that can sequence

Table 3. Different diseases and related circular RNAs that can be used as their probable therapeutic potential

Sl no.	Different diseases	Related circular RNAs (probable therapeutic potential)	Remark/inhibition/regulation type	References
1	lung cancer	cirITCH	downregulation	135
2	gastric cancer	hsa_circ_002059	downregulation	136
3	hepatocellular carcinoma	circ_0067934	–	137
4	cervical squamous cell carcinoma	circCDKN2B-AS1	interacts with IMP3	138
5	renal cell carcinoma	circRNA_001287	targets through miR-144-targeted CEP55	139
6	bladder carcinoma	hsa_circRNA_100876	interacts with mir-136-5p, and with mRNA-chromobox 4 (CBX4) subsequently	140
7	coronary artery disease	hsa_circ_0124644	upregulated	141
8	alzheimer's disease	ciRS-7	upregulates UBE2A	77
9	diabetes	ciRS-7	inhibits miR-7 function in islet β cells	142
10	allergic asthma	hsa_circ_0002594	upregulated in T helper 2-mediated allergic asthma	143
11	schizophrenia	hsa_circRNA_104597	downregulated	144
12	metabolic dysfunction-associated fatty liver disease (MAFLD)	circScd1	upregulated and circScd1 influences JAK2/STAT5 signaling	145
13	hepatocellular carcinoma (HCC)	has_circ_0078710	upregulated and interacts with miR-31/HDAC and CDK2 signaling	146
14	hepatocellular carcinoma (HCC)	circMAT2B	upregulated and interacts with miR-338-3p/PKM2 signaling	147
15	fibrosis	mmu_circ_34116	downregulation; increases α-SMA expression	148
16	fibrosis	hsa_circ_0070963	downregulation; regulates miR-223-3p/LEMD3 signaling	149
17	fibrosis	circMTO1	downregulation; regulates miR-17-5p/Smad7 signaling	150
18	fibrosis	circ-PWWP2A	upregulated; regulates miR-203 and miR-223/TGF-β and LPS signaling	151
19	axial spondyloarthritis	hsa_circ_0079787	it is positively correlated with the plateletcrit (PCT) and negatively correlated with the mean platelet volume (MPV)	152
20	atherosclerosis	hsa_circ_0003575	upregulated; regulates oxLDL-induced vascular endothelial cells	153

full-length circRNA isoforms. The strategy involves rolling circle amplification (RCA) followed by a nanopore long-read sequencing. The strategy successfully prepared a comprehensive catalog of full-length circRNA isoforms from 12 human tissues and one human cell line (HEK293).[159] Another study utilized nanopore technology for long-read sequencing of circRNAs from the brain of mice and humans. The study concluded that for mapping the specific exon composition of circRNA, the nanopore sequencing technology is fast and reliable.[160] Recently, to assemble and quantify circRNAs, an algorithm called circRNA identifier (CIRI) has been developed. CIRI is a group of software that includes CIRI-AS and CIRI-long. CIRI-AS utilizes a spliced junction signature-based algorithm for high-throughput detection of internal components of circRNAs based on short-read sequencing, whereas CIRI-long uses a high-throughput approach to construct full-length circRNAs and circular isoforms. CIRI-long has the advantage that other than providing complete sequences of circRNAs, it permits the analysis of circRNAs at the isoform level. Hence, by utilizing CIRI-long or CIRI-full, differences among the circRNAs at the isoform level were studied in human liver tumor and normal tissues.[161] Another study also utilized CIRI-long for analyzing the data from full-length sequencing of circRNA isoforms using nanopore technology. The strategy was able to identify a new type of circRNA (intronic self-ligated) that exhibited unique splicing and expression patterns.[162]

Recent research progress on the disease association, progression, and therapeutic potential of circRNAs

Recently, a cancer-specific circRNA database was created that contains several identified circRNAs from normal samples as well as both tumor samples. For each circRNA, researchers have predicted RBP sites. Researchers have also predicted the splicing events in each circRNA to understand the association between backsplicing and linear splicing.[163] Another study by Chen et al.[164] found that one intronic circRNA developed from the *AGO2* gene (circAGO2) regulates cancer progression. At the same time, they found that the interaction between circAGO2 and HuR (human antigen R) may

facilitate the activation of circAGO2. Sun et al.[165] found a circRNA-based next-generation biomarker of gastric carcinoma, hsa_circ_0000520. Yu et al.[166] observed that a circRNA called cSMARCA5 regulates metastasis and progression of HCC, and this circRNA was generated from the *SMARCA5* gene (exons 15 and 16). Chen et al.[167] discovered a next-generation biomarker for the diagnosis of gastric cancer, i.e., circRNA hsa_circ_0000190. Recently, circRNA circUHRF1 was observed to be associated with oral squamous cell carcinoma. This circRNA is derived from splicing factors and controls tumorigenesis through a feedback loop.[168] Similarly, circRNA and circ-CCAC1 were observed to have a role in cholangiocarcinoma progression and to have a role in metastasis and tumorigenesis. This circRNA can be a therapeutic target or next-generation biomarker for this disease.[117] Similarly, Xu et al. found another circRNA for this disease that was downregulated. This circRNA controls cell invasion, cell migration, and cell proliferation in cholangiocarcinoma.[169]

FUTURE PERSPECTIVES AND CONCLUSIONS

Just some peculiarities or errors of pre-mRNA splicing do not form circRNAs, but instead, they are tightly regulated transcripts. In some instances, circRNA can perform critical biological functions. Many recent studies in the area of circRNA have enlightened RNA biology in a new direction. circRNAs, unlike linear mRNAs, can possess different functionalities due to their circular dimensions. With the discovery of novel circRNAs, many diverse functions have attracted the attention of scientists. circRNAs are critical players in the appearance of different diseases such as cancer, diabetes, neurological diseases, cardiovascular disease, and many other diseases. Mainly, circRNAs can act as a promising biomarker and have a significant role in therapeutic purposes for different diseases. This emerging area could further show the way for functional diagnosis and successful treatment. Based on unique structural features, biological properties, and biological functions, circRNAs have become the modern research hotspot today, especially in the areas of effective diagnostics and therapeutics. However, more research in this direction is needed to establish the therapeutic potential and use of circRNAs.

More functional characterization can be studied by designing exclusive modern tools such as bioinformatics tools for circRNAs, where the possible target identification and interaction of circRNAs with their possible targets can be studied very efficiently. However, many bioinformatics tools with different signature algorithms are needed that can efficiently detect circRNAs, their structures, and their interactions. Moreover, strategies that will allow determining spatial and temporal gene expression patterns in specific cell types, specifically in single cells, can be more realistic for understanding the biological functions of circRNAs. Also, the development of new and rapid genome-wide circRNA sequencing techniques are required in the near future to annotate the roles and biological functions of circRNAs.

Another intriguing fact is that the secondary structure of circRNA may affect miRNA sponging ability. However, more detailed studies are needed on miRNA sponging. circRNAs in developmental biology should be explored as another area of research. Apart from this exploration, many areas need to be investigated to validate the activities and regulation of circRNAs in both plant and animal systems. However, state-of-the-art methodologies such as CRISPR-Cas9 technology will help in more in-depth research and will help to solve the different research questions.

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AUTHOR CONTRIBUTIONS

A.R.S., M.B., and C.C. researched data for the article. A.R.S., M.B., and C.C. wrote the draft manuscript, substantively contributed to the discussion, and edited the article. S.B. and A.S. contributed to generating the tables and editing and reviewing the manuscript. S.-S.L. and C.C. reviewed and edited the manuscript. A.R.S. and S.-S.L. managed funding and provided management.

DECLARATION OF INTERESTS

The authors declare no competing interests.

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