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REVIEW

Circular RNAs Fifty Years After Their Discovery

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Abstract—Circular RNAs (circRNAs) are a unique class of covalently closed molecules formed through non-canonical splicing and characterized by a markedly greater stability compared to linear RNAs. Although the first circRNA was discovered half a century ago in 1976 in a viroid, they had remained largely overlooked for several decades. Over the past ten years, the however, interest in circRNAs has grown substantially, even as their biological functions and overall significance continue to be debated. It is now well established that circRNAs constitute a large and diverse group of molecules with varied origins and properties. They have been identified across a wide range of organisms, from prokaryotes to plants and mammals, where they participate in the regulation of numerous cellular processes. The unique properties of circRNAs are beginning to be exploited for practical applications, including their use as disease biomarkers and platforms for the development of novel therapeutic strategies. This review summarizes the knowledge accumulated on circRNAs since their discovery and highlights recent advances in understanding their biology and potential applications.

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INTRODUCTION

Circular RNAs (circRNAs) are covalently closed, circular long non-coding RNA (lncRNA) molecules. They were first described in 1976 by Heinz Ludwig Sänger and colleagues, who deciphered the structure of the potato spindle tuber viroid (PSTVd), originally discovered five years earlier by Theodor Otto Diener, and introduced the term "circular RNA" (circRNA) [1, 2]. By the late 1970s, circRNAs had been found in several satellite viruses, including hepatitis delta virus (HDV). The HDV genome was the first circRNA isolated from a human organism [3-5].

Over the past half-century, the accumulation of knowledge about circRNAs has been highly uneven. During the 1980s-1990s and into first decade of the 21st century, less than 200 articles had been published on this topic (PubMed), most of which were focused on isolated examples of individual circRNAs in specific eukaryotic organisms, e.g., the freshwater ciliate *Tetrahymena*, slime mold, Chinese hamster ovary cells, monkey CV-1 cells, and human HeLa cells [6-9]. During the first 35 years following their discovery, up to 2011, circRNAs had remained largely outside the mainstream of molecular biology research. This situation has changed dramatically over the past 15 years, primarily due to the advent of whole-transcriptome sequencing technologies. These approaches have uncovered an unexpected abundance and diversity of circRNAs, particularly in human cells. As a result, more than 25,000 articles published during this period (PubMed) have substantially expanded our understanding of circRNA biogenesis, structure, and biological functions, firmly establishing circRNAs as biologically important molecules.

CircRNAs originate from a wide range of genomic sources. They can be generated from different functional DNA regions, including exons (exonic circRNAs) [10, 11], introns (intronic circRNAs) [12], and combined exon–intron sequences (exon–intron circRNAs,

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EIciRNAs) [13]. In addition, circRNAs may arise from genomic regions involved in gene or chromosomal translocations, giving rise to fusion circRNAs [14, 15], mitochondrial DNA (mitochondrial circRNAs, or mecciRNAs) [16], and tRNA introns, producing circular tRNA introns (tricRNAs) [17]. CircRNAs have been identified across a broad spectrum of organisms, including humans, rodents, and other mammals [11, 18-20], birds [21], fruit flies (*Drosophila* spp.) [22, 23], amphibians [24], as well as plants [2, 25] and even archaea [18].

CircRNAs have attracted considerable attention due to their unique and biologically valuable properties. First, due to the lack of free 5′ and 3′ ends, circRNAs are more metabolically stable compared to linear RNAs [12, 26]. Second, although circRNAs frequently originate from protein-coding genes, they generally do not encode proteins and are regulated by expression mechanisms distinct from those governing messenger RNAs (mRNAs) [22, 27, 28]. Third, circRNAs are particularly abundant in brain tissue [11, 20, 29, 30]. Fourth, numerous studies have demonstrated that circRNAs are actively expressed under a wide range of pathological conditions [28, 31-34]. In recent years, substantial progress has been made in understanding the functional roles of circRNAs. One of the best-characterized functions is their ability to interact with microRNAs (miRNAs), sequester them, and thereby alleviate miRNA-mediated repression of protein-coding transcripts [35-38]. Through the involvement of specific competitive circRNA–miRNA–mRNA regulatory axes, circRNAs can modulate key cellular processes, including cell differentiation, proliferation, invasion, and metastasis in cancer [39-41], as well as viral infections, including COVID-19, and antiviral immune responses [42]. Extensive research has also been focused on the role of circRNAs in cardiovascular diseases, particularly in regulating the blood–brain barrier permeability, limiting ischemic injury, and promoting neuroprotection [36, 37, 43-45]. The substantial regulatory potential of circRNAs, which has been long underestimated, provides grounds for their potential use in biomedical applications. Emerging studies describe the use of circRNAs as disease biomarkers, development of strategies for targeted circRNA delivery as therapeutic agents, creation of novel circRNA-based vaccines, and other advanced therapeutic approaches [46-48].

Currently, two terms are used in Russian-language literature to denote covalently closed RNA molecules: *“циклические РНК”* and *“кольцевые РНК”* (ring-like RNAs). The term *“кольцевые РНК”* was used on the Biomolecula website in 2018 (https://biomolecula.ru/articles/vlast-kolets-vsemogushchie-koltsevye-rnk [in Russian]), as well as in the article by Duk and Samsonova in 2021 [49] and in the review by Baulina et al. in 2024 [50]. In contrast, the term *“циклические РНК”* was first introduced by our group in 2016. This term was chosen as the most linguistically and conceptually consistent with the English-language term “circular RNAs” and has remained in active use since then [51-56].

According to PubMed, about 4000 review articles on circRNAs have been published worldwide, providing comprehensive overviews of these molecules, their functions, and their potential roles in the pathogenesis of various diseases. In contrast, in the Russian-language scientific literature, circRNAs have been described in only a limited number of publications. In the present review, focused primarily on the studies of Russian researchers, we provide a comprehensive overview of the structural organization of circRNAs, their expression features, functional roles, and prevalence. For the first time, information on prokaryotic circRNAs is presented, including viroid-like RNAs known as obelisks. The review discusses recent advances and current trends in the development of practical applications of circRNAs, including their use as disease biomarkers and therapeutic agents, as well as potential applications in forensic science and genome editing technologies. In addition, we present the most significant results of our own studies on circRNAs in brain cells. These include the discovery and characterization of the structural and functional organization of circRNAs derived from the human sphingomyelin synthase 1 gene (*SGMS1*) and its animal homolog (*Sgms1*), as well as the analysis of circRNA expression patterns in cerebral ischemia. We believe that this review will be of interest to specialists in biochemistry and researchers working in related fields of science.

CircRNAs IN CELLS

CircRNA formation *in vivo*. The main stages of circRNA biogenesis have been described in numerous reviews [19, 33, 46, 50, 57, 58]. A key event in circRNA formation is alternative splicing, during which precursor RNAs are processed into either linear or circular RNA molecules. CircRNAs are frequently derived from protein-coding genes and are often generated concomitantly with mRNA production. CircRNA biogenesis occurs through a noncanonical splicing mechanism known as backsplicing. This process involves the joining of a downstream 5′ splice donor site to an upstream 3′ splice acceptor site, which is enabled by the formation of looped RNA structures. Such loops are typically formed by intronic sequences flanking the circularized exon(s). Loop formation can be mediated by base pairing between inverted repeat elements, such as *Alu* elements in primates

or *B1* and *B2* elements in rodents, which are commonly present within introns [11, 59-61]. In the absence of inverted repeat elements, circRNA formation can be facilitated by RNA-binding proteins. For example, proteins such as QKI [62] and FUS [63] can dimerize after binding to specific intronic motifs flanking the circRNA-forming region and bring the backsplicing sites into close proximity, thereby promoting circularization. Studies in *Drosophila* have demonstrated that the efficiency of backsplicing is influenced by the activity of canonical pre-mRNA splicing. Thus, depletion of spliceosome components leads to a substantial increase in steady-state circRNA levels. This phenomenon is thought to arise because circRNA formation requires fewer splicing factors than linear splicing. Similarly, circRNA production is enhanced when 3′-end processing of pre-mRNA is inhibited. Reduced polyadenylation activity can result in the transcriptional read-through across adjacent genes, producing unusually long transcripts that impose increased demand on the splicing machinery. Under these conditions of limited splicing factor availability, circRNAs gain an advantage and become the predominant products of pre-mRNA splicing [22, 27].

The formation of circRNAs is still debated, with some studies interpreting them as “splicing noise” and associating their presence with background mRNA isoforms [64]. Because the structures of many circRNAs have been inferred primarily through bioinformatic analysis of short reads from high-throughput RNA sequencing, their actual existence in cells has been questioned. Technical limitations at multiple stages, including circRNA enrichment, RNA-Seq library preparation, and bioinformatic analysis, can lead to the identification of artifactual circRNAs [65]. Increasing the reliability of circRNA detection requires the use of complementary experimental approaches, such as PCR and Northern blotting [66]. Using these methods, we detected and confirmed several circRNAs derived from the *SGMS1* gene, predominantly composed of exons from the 5′ untranslated region (5′ UTR) [11]. Moreover, *SGMS1* circRNAs exhibited evolutionary conservatism and tissue-specific expression, supporting the view that their formation is a non-random process [20, 67].

It is believed that circRNAs do not require their own promoters for synthesis. However, in the study of circRNAs derived from the rat *Sgms1* gene, we demonstrated that the use of alternative gene promoters can regulate circRNA accumulation. The *Sgms1* gene is transcribed from at least three promoters. At an early stage of rat embryonic development (7 days *in utero*), the majority of transcripts in the embryonic brain originated from the internal promoter. These transcripts retained an intact open reading frame and all elements required for mRNA translation, enabling the production of sphingomyelin synthase 1 protein. However, they lacked the extended 5′ UTR, whose exons were included in the *Sgms1* circRNAs. Consequently, transcription from the internal promoter did not support circRNA formation. At later stages of embryonic development (11-21 days *in utero*) and in 2-month-old adult rats, mRNA expression predominantly originated from distal promoters. This shift was paralleled by the increase in the circRNA level, suggesting that circRNA biogenesis can be regulated through the use of alternative gene promoters.

Diversity of circRNAs. As a result of alternative splicing, polyadenylation, and use of alternative promoters, several classes of circRNAs are generated, including exonic, intronic, and EIciRNAs (Fig. 1). Each class exhibits distinct structural and functional characteristics. Thus, exonic circRNAs are true circular molecules formed by canonical 3′-5′ phosphodiester bonds. In addition to circRNAs derived from exons of a single gene, exonic circRNAs can also arise as chimeric molecules composed of exons from different genes. Such fusion circRNAs may result from chromosomal translocations or read-through transcription of downstream genes [14, 15, 22, 27]. The presence of such chimeric circRNAs is often indicative of ongoing oncogenic processes. Intronic circRNAs are typically lariat-like structures containing an atypical 2′-5′ phosphodiester bond. These molecules can be experimentally distinguished from true circRNAs by sequential treatment with the RNA lariat debranching enzyme (DBR) and RNase R. DBR cleaves the 2′-5′ bond, converting the lariat into a linear RNA, which is subsequently degraded by RNase R, whereas true circRNAs remain resistant to both enzymes [12, 26]. Intronic circRNAs may also be generated through the self-splicing of group II introns with intrinsic ribozyme activity [68]. EIciRNAs contain both exon sequences and intronic regions [13]. In some instances, circRNAs do not encompass entire introns but instead include specific intronic segments referred to as internal recursive exons. These elements participate in the stepwise splicing of exceptionally large introns, sometimes spanning tens of thousands of nucleotides, and can be retained within circRNA molecules [69]. Although recursive exons may be preserved between canonical exons, transcripts harboring them are frequently targeted for degradation through the nonsense-mediated decay pathway [70, 71]. Previously, we identified six circRNAs derived from the human *SGMS1* gene that contain recursive exons. Interestingly, these recursive exons were not only positioned between canonical exons in an order consistent with linear mRNA but also directly participated in backsplicing [69]. Based on these observations, we proposed a model of recursive backsplicing in which circularization occurs between a canonical exon

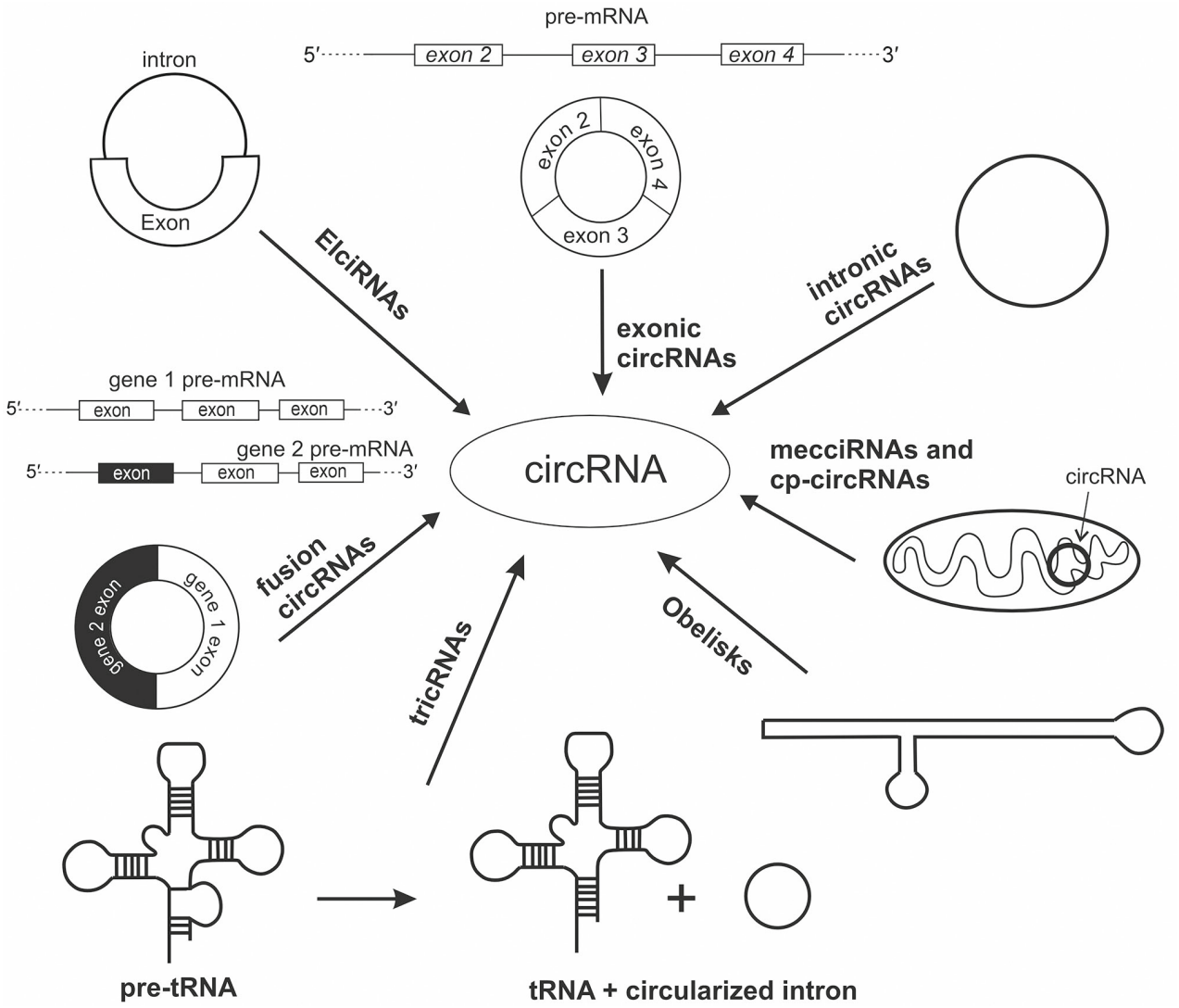

Fig. 1. CircRNA origin: circRNAs are synthesized in different cellular compartments and are derived from exons of one or more genes, introns, tRNA precursors, and obelisks.

and a recursive exon, followed by excision of the intron from the circular precursor [69].

Another conserved mechanism of RNA circularization has been identified in archaea and eukaryotes. tRNA intron lyase (also known as tRNA splicing endonuclease, TSEN) cleaves intron-containing precursor tRNAs at the characteristic bulge–helix–bulge (BHB) motif. Following the cleavage, the exon ends are ligated by specific ligase complexes to generate mature tRNA. In animals, the intron ends can also be ligated to form circular RNAs derived from tRNA introns, known as tRNA intronic circular RNAs (tricRNAs). However, this process is not typical in yeast and plants [17, 72] (Fig. 1). It has been proposed that maintaining tricRNAs in plants and yeast would incur high energy costs, whereas degradation of tRNA introns may help conserve both energy and nucleotide resources [72].

Recently, circRNAs encoded by mitochondrial and chloroplast genomes have been discovered (Fig. 1). Hundreds of mitochondrial genome-encoded circRNAs have been found across various cells and tissues in humans, mice, and other species. These molecules, termed mitochondrial-encoded circRNAs (mecciRNAs), are synthesized from mitochondrial DNA templates through the activity of nuclear-encoded splicing factors imported into mitochondria from the nucleus. Notably, mecciRNAs can facilitate the transport of certain proteins into mitochondria, as they are capable of passing through the mitochondrial pores [16]. In plants, chloroplast genome-encoded circRNAs (cp-circRNAs) have been identified and characterized in *Arabidopsis thaliana*. Sequence analysis suggests that cp-circRNAs may participate not only in plant development, but also in responses to environmental stimuli [73]. Although the precise mechanism of

cp-circRNA biogenesis remains unclear, it is thought to be associated with splicing. Plastid genomes, including chloroplast DNA, are known to contain introns, and gene expression in plastids is subject to extensive post-transcriptional regulation [74, 75].

Unlike in eukaryotes, circRNA formation in bacteria occurs independently of splicing. A study in *Bacillus altitudinis* demonstrated that specific 3′-terminal sequences of linear RNA precursors play a crucial role in the circularization of the *DuCS* (dual-conformation small) non-coding RNA that regulates oxidative stress resistance in this bacterium. Mutations within these terminal sequences abolished the circRNA formation [76]. It was proposed that *DuCS* circularization may involve ribonucleases, including 5′-3′ exoribonucleases and/or site-specific endoribonucleases, together with bacterial RNA ligases. However, the precise molecular mechanism of circRNA biogenesis in bacteria remains to be elucidated [76].

In 2024, Zheludev et al. [77] discovered a new class of viroid-like RNAs, named obelisks, in metatranscriptomes of human gut microorganisms and oral microbiota (Fig. 1). These elements are approximately 1000 nucleotides in length and adopt a rod-like secondary structure reminiscent of viroids. However, unlike classical viroids, obelisks encode proteins of the Oblin family (Oblin-1, Oblin-2, Oblin-SS, etc.), which places them closer to viruses. Oblin-1 contains an RNA-binding domain and is structurally conserved across diverse obelisks, whereas Oblin-2 has a propensity to form protein polycondensates. Some obelisks also exhibit type III hammerhead ribozyme self-cleavage activity [77]. Subsequent studies confirmed that obelisks are rather common. In *Streptococcus sanguinis* SK36, the content of obelisk RNAs can exceed that of cellular mRNAs [78]. In 2025, López-Simón et al. [79] identified dozens of additional obelisks through the analysis of marine metagenomic and metatranscriptomic datasets. The authors proposed that obelisks represent an evolutionary intermediate between viroids and viruses, combining the features of both types of organisms. Studying them may provide new insights into RNA-mediated regulation in microbiomes and shed light on the evolution of pre-cellular life forms.

Several databases (e.g., circAtlas, circBank, and circBase) provide comprehensive information on circRNAs in different species. In Russia, a user-friendly automated Unix/Linux pipeline called CircParser was developed for the annotation of circRNAs from both local and public databases, such as the National Center for Biotechnology Information (NCBI). This tool integrates outputs from widely used *in silico* circRNA prediction programs (CIRI, CIRI2, CircExplorer2, find_circ, and circFinder) and classifies circRNAs according to their structural features (exonic, intronic, exon-intron, or intergenic) based on genome annotation files [80]. Application of CircParser enabled the identification of myogenic circRNAs in Nile tilapia [81].

Additionally, a web-based platform, CircPrime, was developed to facilitate the design of PCR primers and optimization of thermocycling conditions for the reliable identification of circRNAs using conventional PCR techniques. The platform is universally applicable for studying multiple biological species whose genome assemblies are available in the NCBI database [82].

CircRNA degradation. The cellular levels of circRNAs, like those of other transcripts, are determined by the balance between their biogenesis and degradation. CircRNAs are often more stable than linear RNAs, which allows them to accumulate in the cells. Nonetheless, several mechanisms exist to reduce their abundance [83]. In the nucleus, circRNAs can be cleaved by RNase H when they form R-loops through interactions with single-stranded DNA [84]. In the cytoplasm, multiple degradation pathways have been identified. One such pathway involves RNase L, an enzyme that participates in the primary immune response by cleaving viral and certain cellular RNAs [85, 86]. CircRNAs frequently form 16 to 26-nucleotide intramolecular RNA duplexes that act as endogenous inhibitors of double-stranded RNA-dependent protein kinase (PKR), and RNase L-mediated circRNA degradation is thought to be required for PKR activation during viral infection [85]. Another degradation pathway targets m^6A-modified circRNAs via an RNase P-dependent mechanism [87]. Structure-mediated degradation has also been described, in which highly structured hairpin motifs in circRNAs are recognized by UPF1 and G3BP1, triggering endoribonuclease-mediated cleavage [88]. Additionally, Hansen et al. [35] reported an AGO2-dependent pathway, where circRNAs are cleaved following recognition by miRNA–AGO complexes. Beyond enzymatic degradation, circRNAs can be removed from cells through the export in exosomes, an alternative pathway for circRNA clearance [47, 89-91].

FUNCTIONAL SIGNIFICANCE OF circRNAs

CircRNA interactions with miRNAs. In 2013, it was demonstrated that the circRNA *Cdr1as* can bind complementarily to the miRNA *miR-7* and inhibit its activity in mammalian cells [35]. Because of this function, *Cdr1as* was also termed *Cirs-7* (circRNA sponge for *miR-7*). The identification of *Cirs-7* sparked extensive research into the role of circRNAs as endogenous miRNA sponges.

miRNAs are a class of short (18-21 nt) non-coding RNAs found in various organisms [92, 93] and

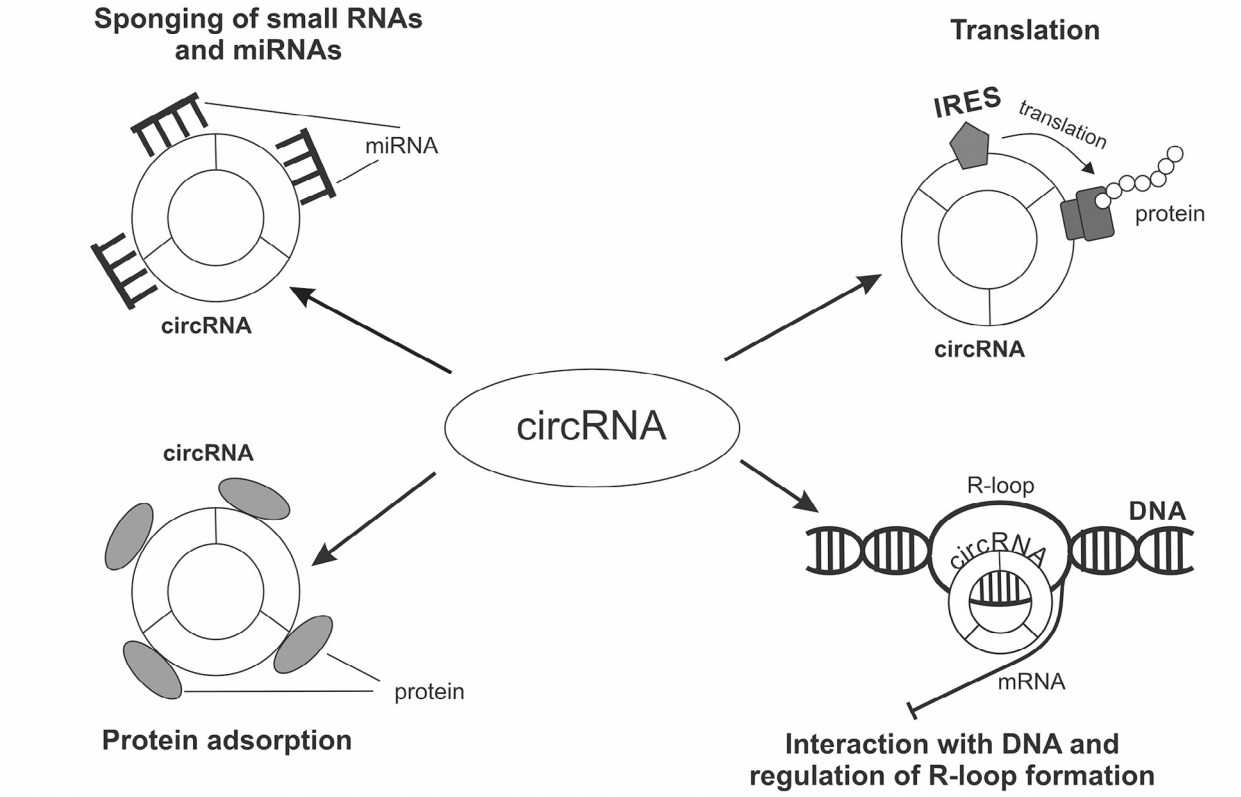

Fig. 2. Biological functions of circRNAs.

involved in post-transcriptional control of gene expression through interaction with mRNAs. In animal cells, this interaction typically occurs via an miRNA sequence complementary to the corresponding mRNA target [94]. The formation of the mRNA–miRNA duplex usually leads to the repression of mRNA translation or its degradation [92]. However, if a circRNA containing a sequence complementary to the miRNA is present, the miRNA can alternatively bind to the circRNA. This sequestration prevents miRNA from interacting with its target mRNA, thereby increasing the levels of both the mRNA and the protein it encodes.

The sponging of miRNAs is considered the primary and most well-established function of circRNAs (Fig. 2). Numerous competitive circRNA–miRNA–mRNA axes have been identified that regulate both normal physiological and pathological processes. Examples include prostate cancer (*circDHRS3*/*miR-421*/*MEIS2*), ischemic stroke (*circHECTD1*/*miR-27a-3p*/*FSTL1*; *circHECTD1*/*miR-133b*/*TRAF3*; *circMap2k1*/*miR-135b-5p*/*Pidd1*), and Alzheimer's disease (*circHDAC9*/*miR-138*/*Sirtuin-1*) [36, 37, 41, 43-45, 95]. It is believed that circRNAs (predominantly exonic) can be transported to the cytoplasm, where they act as competitive endogenous RNAs. Recent studies have elucidated the transport mechanism, which involves Ran–GTP, exportin-2, and IGF2BP1 [96].

It is important to note that circRNAs can compete with mRNAs and lncRNAs in regulating miRNA levels through the target RNA-directed miRNA degradation (TDMD) on mRNA or lncRNA templates, as demonstrated for *Cdr1as* [38, 97]. The TDMD mechanism has recently attracted significant research interest, and its underlying details, including the contribution of circRNAs, are currently the subject of active investigation. Recent studies indicate that efficient miRNA binding to circRNA sequences depends on the presence of multiple corresponding binding sites and a high level of circRNA expression [49]. Establishing and validating the role of circRNAs as sponges for miRNAs requires a multifaceted approach, combining bioinformatics analyses with experimental methods, including dual-luciferase reporter assays, RNA precipitation techniques such as RNA pull-down (RPD) and RNA immunoprecipitation (RIP), as well as circRNA overexpression and knockdown [98]. Several databases provide information on miRNA–circRNA interactions. For example, CircFunBase offers data derived both from bioinformatic predictions and experimental validation [99]. CircInteractome focuses on predicted binding sites and includes tools for designing primers that selectively amplify circRNAs in PCR, avoiding linear RNAs [100]. ENCORI (formerly starBase) provides interaction data obtained through immunoprecipitation methods and also allows prediction

of whether an miRNA may undergo degradation via the TDMD mechanism upon interacting with a circRNA [101].

It should be noted that many reported circRNA–miRNA interactions are derived from single studies and are not always supported by a robust evidence base, which may raise questions about their biological significance. Moreover, circRNAs exhibit diverse functional roles beyond acting as sponges for miRNAs, thus emphasizing the complexity of these molecules as research subjects.

Functions of circRNAs in the nucleus. In addition to their cytoplasmic roles, circRNAs exhibit important nuclear functions. For instance, circular intronic RNAs (ciRNAs) have been shown to regulate transcription by interacting with the RNA polymerase II complex [12, 102, 103]. Similarly, EIciRNAs can associate with RNA polymerase II and small nuclear RNAs (snRNAs) due to the presence of snRNA-binding sites within their retained introns [13]. These examples highlight that many circRNA functions are predominantly nuclear in nature. The interest in nuclear circRNAs has grown significantly in recent years [104, 105]. One notable function involves the prevention of R-loop formation [84]. R-loops occur when a strand of genomic DNA hybridizes with its nascent RNA transcript, which can interfere with the pre-mRNA post-transcriptional processing. Cells typically mitigate R-loop accumulation through the activity of helicases such as DHX9 and DDX5 [106] or the enzyme RNase H [107]. While RNase H can resolve R-loops by cleaving RNA, this often results in the degradation of pre-mRNA and consequent decrease in gene expression. It was shown that circRNAs can compete with the synthesized RNA for the binding to the single-stranded DNA in the R-loop. RNase H recognizes the DNA–circRNA hybrid and cleaves the circRNA, eliminating the R-loop without damaging the pre-mRNA, thereby preserving gene expression [84, 108].

The ability of circRNAs to interact with DNA had formed the basis for the 2022 hypothesis of eco-crossover, also termed circRNA-regulated metabolic crossover, that was suggested by the renowned theoretical biology scientist A. M. Olovnikov [109]. According to this hypothesis, various stress factors can alter the expression of specific genes and their associated circRNAs. As the levels of these circRNAs increase, they may influence chromatin architecture, thereby modulating the transmission of hereditary information. Several intrinsic properties of circRNAs support this idea: their enhanced metabolic stability, stress-responsive expression, presence in germline tissues [110, 111], and capacity for intercellular transfer via exosomes or other extracellular vesicles [47, 89, 90]. Based on these features, Olovnikov proposed that circRNAs could function as adapters targeting specific genomic sequences, facilitating recombination and potentially guiding non-random mutagenesis under environmentally regulated conditions [109].

CircRNAs interactions with proteins. Various interactions between circRNAs and proteins have been described. The resulting circular ribonucleoprotein complexes (circRNPs) perform diverse functional roles in cellular processes [112, 113] (Fig. 2). For instance, circRNAs were found to directly interact with transcription factors in many forms of cancer. A well-characterized example is *Cdr1as*, which interacts with the tumor suppressor and transcription factor p53 at a region critical for p53 binding to the MDM2 protein. Normally, MDM2 represses p53 activity, thus facilitating cancer cell proliferation. By binding to p53, *Cdr1as* prevents the formation of the p53–MDM2 complex, thereby stabilizing p53 function and enhancing its tumor-suppressive activity. Conversely, inactivation of *Cdr1as* contributes significantly to tumorigenesis, highlighting its anti-oncogenic role [114]. In contrast, *circRHOT1* exhibits a pro-oncogenic function. It binds transcription factors and facilitates their recruitment to the promoter of the *NR2F6* gene. Expression of *NR2F6* in T cells suppresses the anti-tumor immune response. Elevated expression of *circRHOT1* has been identified as a negative prognostic factor in hepatocellular carcinoma [115].

There is also evidence that circRNAs are present in RNP complexes involved in the epigenetic regulation of gene expression. For instance, *circFECR1* has been identified in a complex that demethylates the promoter of the leukemia virus *FLI1* gene [116]. In contrast, *circLRP6* promotes DNA methylation and functions as an oncogene. This circRNA interacts with LSD1 and EZH2 proteins, thereby enhancing methylation and reducing expression of tumor suppressor genes such as *KLF2* and *APC* that normally act to slow osteosarcoma progression [117]. In all the above cases, the interactions between circRNAs and their associated proteins have been experimentally validated.

Another notable property of certain circRNAs is regulation of the primary immune response, in which the cell detects viral infection and activates a system to degrade foreign nucleic acids through a network of receptors and nucleases. During this process, specific circRNAs, e.g., *circPOLR2AP*, are produced that bind to NF90 and NF110 proteins (co-factors in immune gene transcription) and sequester them in the cytoplasm. In the absence of infection, these proteins remain bound to circRNAs and are inactive. Upon viral infection, circRNAs are rapidly degraded by RNase L, releasing NF90 and NF110 to interact with viral RNA and trigger the immune response. Additionally, under normal conditions, these proteins promote the

formation of corresponding circRNAs in the nucleus, creating a reservoir that is ready for rapid deployment upon subsequent infections [118-120].

Functional interactions with proteins have also been reported for mecciRNAs in humans, mice, and other species. These circRNAs can be transported from mitochondria to the cytoplasm, where they participate in the transport and processing of nuclear proteins, such as RPA (replication protein A) and hnRNPA (heterogeneous nuclear ribonucleoprotein A), which are critical for the mitochondrial genome replication and transcription [16]. MecciRNAs have also been implicated in cancer progression and resistance to anticancer therapies [121].

A recent study revealed that circRNAs play a key role in modulating cellular responses to heavy metal-induced stress. Central to this response is the RNA-binding protein gawky, which translocates to the nucleus and functions as a chromatin-associated factor, activating the transcription of numerous stress-responsive genes. In the case of copper-induced stress, gawky has been shown to interact with circRNAs containing metal-sensitive elements. Overexpression of these circRNAs suppresses stress-induced transcription by promoting the dissociation of gawky from the chromatin [122]. Therefore, circRNAs can act as negative regulators of stress-responsive gene expression, impairing the chromatin-dependent activity of the stress-activated gawky protein.

Translation of circRNAs. For a long time, circRNAs had been classified as lncRNAs. However, emerging studies indicate that some endogenous circRNAs have a potential for translational, which can be initiated by N6-methyladenosine (m^6A), the most common RNA base modification, the presence of internal ribosome entry sites (IRESs), or AU-rich motifs (Fig. 2). CircRNAs have been shown to produce both full-length proteins [123-125] and small peptides [126-128]. Certain RNA-binding proteins facilitate cap-independent translation by recognizing motifs in the circRNA sequence that resemble IRES, exhibiting varying specificity and affinity for these sites [129]. Moreover, recent evidence suggests that most eukaryotic initiation factors (eIFs) are also essential for circRNA translation [130]. Several bioinformatics tools, such as TransCirc, RiboCirc, CircAtlas, and CRAFT, can predict circRNAs with the coding potential [131]. A collaborative effort involving Russian researchers has led to the creation of the AthRiboNC database, which catalogs non-coding RNAs, including circRNAs with the coding potential, in *A. thaliana*. This database is based on ribosome profiling data (Ribo-Seq) and contains information on 1871 circRNAs. Its interface allows alignment with nucleotide or amino acid sequences, as well as search and filtering by genomic location, expression level, and other criteria [132].

CircRNAs as participants in low-affinity nucleic acid interactions. Molecular recognition between nucleic acids is traditionally thought to rely on complementary base-pairing. However, recent study by Nikitin [133] has described the phenomenon of strand commutation – a reversible, low-affinity binding of essentially non-complementary strands. Nikitin demonstrated *in vitro* that the role of such low-complementary interactions can be similar to that of classical complementary interactions. Specifically, the addition of a single-stranded DNA with a limited complementarity to the target DNA significantly altered the efficiency of DNA cleavage by RNase H [133]. This effect can be explained by the competition between low-complementary nucleic acids, consistent with the principles of reversible reactions. These findings are particularly relevant for understanding the functions of non-coding RNA, including miRNAs and circRNAs. CircRNAs are highly stable compared to linear RNAs due to their resistance to nucleases, suggesting they may play a substantial regulatory role even in low-affinity interactions. For instance, miRNAs are approximately 20 nucleotides long but typically recognize RNA targets through short 6-8 nucleotide sequences. CircRNAs may influence miRNA target selection, thereby modulating their regulatory impact. Moreover, circRNAs could interact directly with DNA, potentially preventing R-loop formation and thereby influencing processes such as RNase H-mediated cleavage (Fig. 2). Competitive interactions involving circRNAs, including low-affinity binding, may therefore significantly affect the outcome of nucleic acid–nucleic acid interactions within R-loops.

CircRNA EXPRESSION IN NORMAL AND PATHOLOGICAL CONDITIONS

Tissue-specific expression of circRNAs. Numerous studies have demonstrated tissue-specific expression of circRNAs. Elevated levels of circRNAs are observed in the heart, liver, and other tissues [134-137]. In addition, circRNAs show developmental stage-specific expression [135, 138] and accumulate in anucleate platelets [139] and exosomes [140-142]. Thus, brain tissues have a high circRNA level [19, 20, 30], with genes involved in neurotransmission, neuronal differentiation, and synaptogenesis expressing the greatest number of circRNAs [11, 20, 29, 30]. Our previous study on circRNAs of the *SGMS1* gene in humans, rats, and mice revealed an increased abundance of these molecules in brain cells [11, 69]. You et al. [143] reported that circRNAs predominantly localize to neuronal regions, including neuropils, axons, and dendrites, with their levels dependent on the synapse development and homeostatic plasticity.

Due to a higher synaptic density, human brains often contain more circRNAs than rodent brains [20, 144]. Expression of circRNAs in the brain can also be cell type-specific. For example, Dong et al. [145] identified 1526 circRNAs in dopaminergic neurons and 3308 circRNAs in pyramidal neurons. Some circRNAs have clear functional roles; for instance, *circTulp4* regulates neurotransmitter release at excitatory synapses and enhances behavioral responses to aversive and anxiogenic stimuli in mice [146].

Moreover, an age-related increase in the circRNA expression has been reported across various organisms [147-151]. In tissues with a high proportion of post-mitotic cells, such as the brain, circRNAs are likely to accumulate over time due to their inherent resistance to degradation. Such elevated circRNA abundance in the brain may result from both enhanced activity of factors that promote circRNA biogenesis, such as QKI (Quaking, KH domain containing RNA binding protein), and reduced activity of factors that inhibit their formation, e.g., ADAR1 (adenosine deaminase acting on RNA 1) [20, 62, 152].

Involvement of circRNAs in disease pathogenesis. In recent years, research on circRNAs has expanded significantly, revealing their crucial role in the pathogenesis of various diseases. Numerous circRNAs have been implicated in cardiovascular conditions, including cardiomyopathy, chronic heart failure, hypertension, ischemic heart disease, and atherosclerosis [98, 153-155]. One of the most studied circRNAs in vascular pathology is *circANRIL*. Its role, however, appears to be context-dependent. In vascular smooth muscle cells and macrophages, high *circANRIL* expression is associated with a reduced severity of coronary artery disease [31]. Conversely, in endothelial cells, *circANRIL* overexpression correlates with an increased atherogenic index, elevated serum lipid levels, and higher expression of inflammatory cytokines (IL-1, IL-6), matrix metalloproteinase-9 (MMP-9), and C-reactive protein (CRP) [156]. Other circRNAs, including *circLrp6*, *circDiaph3*, *circCHFR*, and *circDcbld1*, contribute to vascular pathologies by regulating key cellular processes, such as proliferation, migration, and differentiation of vascular smooth muscle cells [157-160]. *CircRNA_000203* (*circMyo9a*) promotes the expression of myocardial fibrosis-related genes through the interaction with *miR-26b-5p* [161]. Similarly, *circRNA_010567* exerts the profibrotic effects via the *circRNA_010567*/*miR-141*/*TGF-β1* regulatory axis.

The increased level of circRNAs in brain cells [11, 20, 29, 30] has highlighted their role as key regulators in various neuroinflammatory and neurodegenerative diseases [162, 163]. Moreover, circRNAs have been implicated in the pathogenesis of glioma, schizophrenia, and autism spectrum disorders [30, 164, 165]. Specific circRNAs, including *circMyst4*, *circKlhl2*, *circAagab*, and *circHomer1*, may contribute to neuroplasticity and synaptogenesis [166]. Several studies have also emphasized the critical role of circRNAs in cellular responses to cerebral ischemia. For instance, Zuo et al. [167] reported that elevated levels of *circFUNDC1*, *circPDS5B*, and *circCDC14A* positively correlate with cerebral infarct volume. Conversely, Bai et al. [37] demonstrated that upregulation of *circDLGAP4* significantly mitigates neurological deficits and protects against infarct expansion and blood-brain barrier damage in a mouse stroke model. Han et al. [36] showed that *circHectd1* regulates regenerative mechanisms in brain cells during ischemia, notably reducing infarct size in ischemic mice. *CircMap2k1* has been identified as a potential contributor to the pathogenesis of cerebral ischemic stroke [45].

Using the transient middle cerebral artery occlusion (tMCAO) model, we characterized the whole-genome array of circRNAs potentially involved in the rat brain response to ischemia in the affected hemisphere [28, 51, 168]. A differential circRNA expression was observed across brain regions with varying degrees of injury, including the striatum and frontal cortex, suggesting involvement of these molecules in regulating cellular responses, including those critical for the functional recovery after cerebral ischemia. Furthermore, we predicted mRNA–miRNA–circRNA interaction networks that may modulate genome activity during ischemia, both within the ischemic focus and in the surrounding penumbral regions containing recoverable cells [28, 51, 169].

The studies on the role of circRNAs in various pathologies can expand our understanding of the molecular processes underlying tissue damage and recovery.

DEVELOPMENT OF circRNA-BASED TECHNOLOGIES

Synthesis of circRNAs *in vitro*. CircRNAs hold considerable promise for biomedical applications, and their potential use in the development of therapeutics, vaccines, genome editing systems, and tools for studying diverse metabolic processes has been actively explored [170]. These prospects highlight the importance of efficient synthetic approaches for circRNA production. CircRNAs can be generated *in vitro* using either chemical or enzymatic methods. Chemical strategies typically rely on the modification of RNA molecule ends to promote circularization. For example, RNA molecules bearing a 3′-amino group and a 5′-phosphate can be circularized using the phosphate-activating reagent 1-ethyl-3-(3-dimethylami-

nopropyl)carbodiimide (EDC). This reaction produces a non-natural phosphoramidate (P–N) bond at the ligation site. Although this linkage does not naturally occur in cells, circRNAs synthesized in this manner are recognized by the cellular translational machinery and can serve as templates for polypeptide synthesis [171]. An alternative chemical approach involves modification of the phosphorylated 5′-end of RNA molecule with a nitrile group, which subsequently reacts with the 3′-hydroxyl group to achieve circularization. However, this strategy frequently generates undesired by-products, including circRNAs containing 2′-5′ phosphodiester linkages. To improve the ligation efficiency and reduce side reactions, single-stranded DNA oligonucleotides complementary to the splice junction can be used to bring the 3′ and 5′ ends into close proximity, thereby facilitating intramolecular ligation. Among chemical approaches, significant attention has recently been directed toward click chemistry strategies and bioorthogonal reactions. These reactions are characterized by rapid kinetics, high yields, and minimal byproducts and involve functional groups that are not naturally present in biological systems and therefore do not interfere with endogenous biomolecules. A commonly employed strategy is azide–alkyne cycloaddition, in which the 3′ and 5′ ends of the RNA are modified with azide and alkyne groups, respectively. Although this method can provide high yields of circular products, it also introduces a non-natural linkage at the ligation site. Chemical ligation strategies have been comprehensively reviewed in [172-176]; however, the advantages and limitations of chemical approaches for circRNA synthesis require further systematic investigation.

Enzymatic approaches for generating circRNAs *in vitro* are primarily based on ligating the 5′ and 3′ ends of linear RNA molecules using RNA ligases. In this method, a 5′-monophosphorylated linear RNA is first produced by *in vitro* transcription of a PCR product fused with the T7 phage promoter, and the DNA template is then removed by treatment with DNase. The 5′ and 3′ ends of the linear RNA are ligated using T4 RNA ligase, and residual linear RNA is subsequently eliminated by digestion with RNase R. Importantly, the choice of ligase may depend on specific nucleotides (A, U, G, or C) present at the ligation junction. To further enhance the ligation efficiency, complementary DNA splints are commonly employed to bring the reactive RNA ends into close proximity, thereby facilitating intramolecular circularization [173, 174, 176].

CircRNA formation can be driven by the catalytic activity of group I introns, which undergo self-splicing. To utilize this capability, an artificial construct is designed in which the sequence intended for circularization (exon) is positioned between two ribozyme-active intron fragments. Specifically, the 3′ half of the intron along with the 3′ splice site is placed upstream of the exon, while the 5′ splice site and the 5′ half of the intron are positioned downstream. During the splicing reaction, group I intron catalyzes excision of the internal exon as a covalently closed circular RNA. This system can be applied both *in vivo* during transcription and *in vitro* to generate target circRNAs [174, 177, 178].

CircRNA overexpression is commonly used in research practice. This approach involves generation of an expression construct in which the circRNA sequence is flanked by artificially designed inverted repeats and placed under control of a strong promoter, such as the cytomegalovirus (CMV) promoter, in a plasmid vector [35, 127]. High levels of circRNA overexpression can also be achieved by cloning the sequence of interest into a tRNA gene driven by a strong promoter. In this strategy, the native tRNA intron is replaced with the target circRNA sequence. During pre-tRNA processing in animal cells, this modified transcript is circularized, leading to the formation of the desired circRNA instead of a tricRNA [17]. The choice of the method for generating circRNAs depends on specific research objectives and intended applications. For example, circRNAs produced using ribozyme-mediated circularization have been shown to support efficient translation in eukaryotic cells [179, 180]. Such constructs have been used in the development of vaccines against SARS-CoV-2 and its variants, where they induced higher proportions of neutralizing antibodies compared to conventional mRNA vaccines [181, 182]. However, ribozyme-derived circRNAs may contain additional sequences, commonly referred to as splicing scars, which can trigger an undesired innate immune response. To address this limitation, a scar-free strategy was developed in which the target sequence is rearranged so that its ends mimic exon sequences required for the intron-mediated splicing [170, 183]. Subsequently, an alternative scar-free approach based on group II introns was introduced that involves modification of the exon-binding site within the D1 domain [170, 184]. Another strategy for the scar-free circRNA synthesis employs *trans*-splicing ribozymes [170, 185]. In addition, circRNAs with a minimal immunogenicity can be generated enzymatically using T4 RNA ligase [186]. When circRNAs are considered as templates for protein synthesis, overexpression systems can be employed to evaluate the translation efficiency and activity of IRESs. However, it is essential to ensure that no *trans*-splicing occurs during circRNA formation from the expression construct. Otherwise, linear mRNA species may be generated, leading to the protein production independently of circRNA [187].

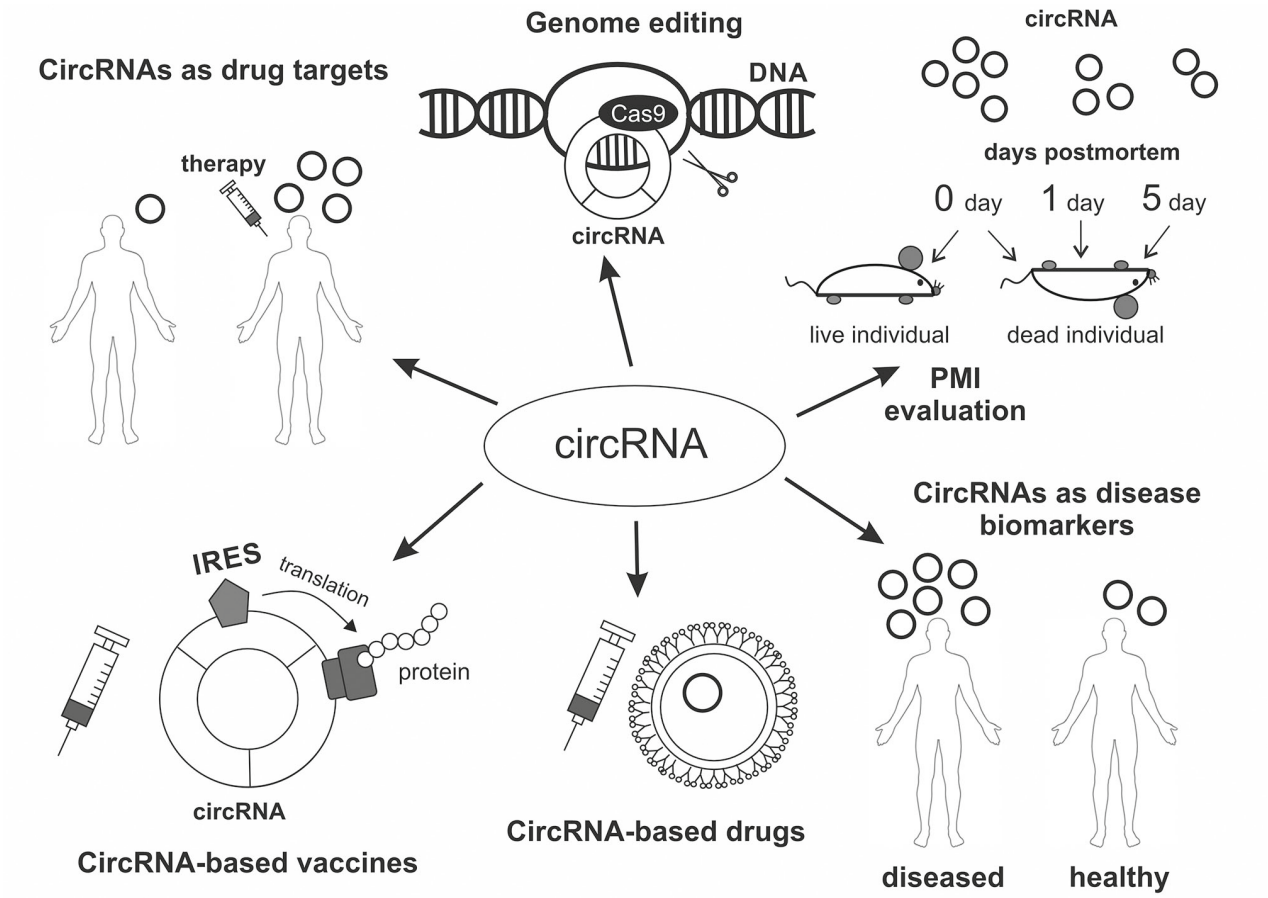

Fig. 3. CircRNA applications.

CircRNAs in genome editing. Genome editing technologies are rapidly advancing, with CRISPR–Cas systems currently representing the most widely used platforms. Guide RNAs (gRNAs) are essential components of these systems, as they direct Cas nucleases to specific DNA or RNA targets for precise modification. However, linear gRNAs exhibit a relatively short half-life compared to Cas proteins, which can substantially limit the overall editing efficiency [188]. Due to their enhanced stability, circRNAs have recently emerged as promising alternatives to linear gRNAs in genome editing applications [189, 190] (Fig. 3). Their covalently closed structure confers resistance to exonuclease-mediated degradation, potentially prolonging guide activity and improving editing outcomes. Recent studies have demonstrated that engineered circRNAs capable of recruiting ADAR enzymes significantly enhance both the efficiency and specificity of RNA editing [189, 190]. In 2023, a research group from China reported the development of a guide circRNAs compatible with the Cas12a and Cas13d systems [191]. CRISPR platforms based on Cas12a and Cas13 offer potential therapeutic advantages over Cas9, including reduced off-target activity and improved suitability for multiplex gene editing [192-194]. Furthermore, in 2025, Zhang et al. [195] demonstrated that guide circRNAs can also be adapted for the use with the compact Cas12f (Cas14) nuclease, which may be particularly advantageous for precise genome editing and biomedical applications.

Recently, circRNAs have been explored for the use in genome editing via the prime editing approach [196-198]. The prime editing system typically consists of a Cas9 nickase fused to a reverse transcriptase and a specialized gRNA known as a prime editing gRNA (pegRNA). This pegRNA not only directs the nickase to the target DNA site but also serves as a template for the synthesis of a new DNA segment during reverse transcription [199, 200]. CircRNAs have demonstrated applicability in CRISPR–Cas12a-based systems [198], in configurations employing separate nickase and reverse transcriptase components [197], and in so-called reverse editing systems that enable modifications at the sites located closer to the 5′ end relative to the nickase cleavage site [196]. Further development of these approaches is expected to substantially broaden the scope of nucleic acid editing, enhancing both the efficiency and translational potential of these technologies in biomedicine.

CircRNAs AS BIOMARKERS

It has been well established that circRNA levels can vary depending on specific pathological conditions, making circRNAs promising biomarkers for various diseases (Fig. 3). The first proposals for developing the panels of circRNA biomarkers, primarily for cancer samples, have already been reported [201-203]. Earlier, we identified circRNAs originating from the *HDLBP* and *TNFRSF1A* genes in RNA extracted from human peripheral blood mononuclear cells. Using bioinformatics analysis, we predicted regulatory networks of competitive interactions between miRNAs and mRNAs or circRNAs for genes involved in lipid metabolism [204]. Upregulation of *circSPARC* and *circTMEM181* has been observed in peripheral blood mononuclear cells of patients with ischemic heart disease compared to individuals without atherosclerosis, supporting the pro-atherogenic role of these molecules [205]. Recent studies indicate that circRNAs can also serve as markers of age and life experience, reflecting the organism's ability to "remember" stress. For instance, exposure of *Drosophila* flies to low (18°C) or high (29°C) temperatures for 10 days induced expression of specific subgroups of circRNAs, whose elevated levels persisted for several weeks even after returning to standard conditions [151].

Establishing regulatory networks involving circRNAs enables the development of test systems that account for the mutual interactions of different RNA types, thus facilitating the selection of RNA panels for more accurate diagnostics with reduced false results. The primary approach relies on the well-established functions of circRNAs, particularly their ability to bind miRNAs. By leveraging individual competitive circRNA–microRNA–mRNA axes, it is possible to modulate global cellular processes, including differentiation, proliferation, invasion, and metastasis in cancer [39-41], as well as coordination of progression of viral infection, such as COVID-19, and antiviral immune responses [42, 206]. Consequently, potential diagnostic systems could be based on the expression levels of circRNAs and their associated miRNAs. Such systems might be highly efficient because circRNAs and miRNAs are stable, exhibit tissue- and disease-specific expression, and circulate in extracellular fluids, making them convenient analytes. For example, the circRNA-to-miRNA ratio (*circR-284*/*miR-221*) has been proposed as a predictive marker for carotid artery disease and stroke [207].

Several databases summarize experimentally validated data on the circRNA expression in various diseases. In 2017, Yao et al. [208] developed the Circ2Disease database, which provided 274 experimentally confirmed associations between the circRNA–miRNA axes and human diseases; however, this database is no longer updated. The circRNADisease database (updated in 2023) contains approximately 7000 entries documenting experimentally confirmed associations of circRNAs with a wide range of human and animal diseases [209]. For instance, the use of "brain ischemia" prompt for searching this database provides information on circRNAs derived from the *CDR1*, *DLGAP4*, *OGDH*, *FUNDC1*, and other genes, detailing their differential expression during ischemia and miRNAs with which they can interact. The database also catalogs over 7 million associations between circRNAs and mutations across 30 cancer types, with the highest number of circRNA mutations observed in endometrial cancer, skin melanoma, and colorectal adenocarcinoma. Additional resources, including MiOncoCirc [210], CircNet 2.0 [211], and CSCD2 [212], provide information on circRNA expression and functions in various cancers. At the end of 2025, Yuan et al. [213] launched the BloodCircR database, which compiles data from RNA sequencing of 5430 human peripheral blood samples associated with 58 diseases. This resource holds significant promise for identifying circRNA biomarkers and circRNAs with a potential therapeutic relevance.

However, circRNA-based test systems may exhibit significant variability. Factors such as the cellular concentrations of proteins interacting with circRNAs or R-loops generated during transcription and replication, should be taken in consideration, as circRNAs have been shown to participate in resolving R-loops [84]. Evidence also suggests the involvement of these mechanisms in various pathological processes. Clearly, in such contexts, different strategies for the quantitative detection of analytes in the corresponding test systems will be necessary. At present, however, these possibilities remain largely in the realm of scientific prospects.

The potential of circRNAs in forensic medicine has been increasingly recognized (Fig. 3). Because of their metabolic stability, circRNAs are promising biomarkers for estimating the postmortem interval (PMI). For instance, the level of *circRnf169* in liver tissue has been suggested as a potential marker for early PMI assessment. However, as noted by the authors, its utility for late PMI estimation is limited due to the dynamic degradation of circRNAs in the liver [214]. To address this limitation, the authors investigated circRNA markers in brain tissue and found that *circFat3* exhibits tissue-specific expression in the mouse brain and demonstrates a strong correlation with PMI over a wide temperature range [215].

Although increasing evidence highlights the potential of circRNAs as biomarkers, reproducibility across different studies remains low. Therefore, further experimental validation is essential to establish circRNAs as reliable components of diagnostic panels.

CircRNAs AS THERAPEUTIC TARGETS

Advances in deciphering the mechanisms by which circRNAs regulate gene expression have opened new avenues for therapeutic interventions. Targeting circRNAs or associated regulatory networks to modulate their activity is a promising strategy for disease treatment. Emerging evidence demonstrates that altering circRNA activity can profoundly reprogram cellular metabolism and function. Such modulation has been shown to affect the blood–brain barrier permeability and infarct size [37], as well as key oncogenic processes, including cell differentiation, proliferation, invasion, and metastasis [39, 216]. Furthermore, circRNAs are involved in pathogenesis of viral infections, including COVID-19, where they contribute to the viral replication dynamics and modulation of antiviral immune responses [42] (Fig. 3). A notable example of circRNA-targeted therapy involves triple-negative breast cancer (TNBC). Approximately one-third of TNBC patients express *circHER2*, a circular RNA encoding the oncogenic protein HER2-103. The therapy of HER2-amplified breast cancer widely uses the monoclonal antibody pertuzumab, which has been shown to significantly reduce the tumorigenicity of cells expressing *circHER2*/HER2-103 *in vivo* [217]. In another study, the authors demonstrated that the circRNA *hsa_circ_0003220* mediated resistance of non-small cell lung cancer (NSCLCs) to chemotherapeutic drugs through regulation of the *miR-489-3p*/IGF1 axis. Suppression of this circRNA reduced the expression of the pro-oncogenic protein IGF1 and restored the sensitivity of tumor cells to therapy [218]. Similarly, the *circ_ZFR*/*miR-195-5p*/*KPNA4* regulatory axis has been identified as a key determinant of paclitaxel sensitivity in NSCLCs, representing a potential therapeutic target [219]. Paclitaxel exerts its antitumor effects by disrupting the microtubule dynamics and mitosis progression, and circRNA-mediated regulation significantly influences cellular responsiveness to this drug. The *hsa_circ_0074027*/*miR-379-5p*/*IGF1* axis has been shown to contribute to the molecular mechanisms underlying chemoresistance in NSCLCs [220]. Therefore, assessment of circRNAs as therapeutic targets is extremely important, as targeted modulation of specific circRNAs or circRNA-dependent signaling pathways may significantly enhance the treatment efficacy [221, 222].

CircRNA-BASED DRUGS

Currently, circRNA-based therapeutics are being actively developed. Particular attention is focused on their potential application in glioma treatment. Accumulating evidence indicates that circRNAs contribute to glioma resistance, primarily by mediating resistance to radiotherapy and chemotherapy. Mechanistically, circRNAs exert these effects by functioning as competitive endogenous RNAs (ceRNAs), regulating miRNA activity within specific signaling axes, including *circATIC*/*miR-520d-5p*/*Notch2-Hey1* [223], *circ-0008344*/*miR-433-3p*/*RNF2* [224], and *circASAP1*/*miR-502-5p*/*NRAS* [225]. Exosome- or lipid nanoparticle-encapsulated *circPRKD3* has been shown to stimulate CXCL10 secretion through the reprogramming of tumor-associated macrophages, thereby enhancing recruitment and tumor infiltration of $CD8^+$ T cell. Recently engineered circRNA encoding interleukin-2 (IL-2) demonstrated significant tumor-suppressive effects in a glioma model [226]. To facilitate its delivery, a specialized lipid nanoparticle system based on ursodeoxycholic acid was developed for efficient circRNA transport.

CircRNAs can be delivered via extracellular vesicles (e.g., exosomes) to target cells, where they exert their protective effects. It was shown that circRNAs are naturally transported in exosomes [91, 140]. Accordingly, one of the most actively developing research areas focuses on characterizing the circRNA cargo of exosomes derived from various sources and elucidating the functional role of these molecules. Yang et al. [47] showed that extracellular vesicle-mediated delivery of *circSCMH1* promotes its interaction with MeCP2, thereby relieving repression of target genes regulated by this protein. This results in a significantly enhanced neuroplasticity, along with reduced glial reactivity and decreased infiltration of peripheral immune cells in rodent and primate models of ischemia. Similarly, exosome-mediated delivery of *circDYM* alleviated chronic unpredictable stress-induced depressive-like behavior in mice [89]. Collectively, these findings highlight the therapeutic potential of exosome-based circRNA delivery as a promising strategy for neuroprotection and treatment of neuropsychiatric disorders (Fig. 3). However, clinical translation remains limited by insufficient understanding of exosome biology, as well as concerns regarding the efficiency and safety of developed technologies. Despite these challenges, combining exosome-based delivery systems with bioactive molecules such as circRNAs may open new avenues for the treatment of complex multifactorial diseases.

CircRNA-based vaccines. The onset of the COVID-19 pandemic has significantly accelerated research into novel RNA-based vaccine platforms. Due to their enhanced metabolic stability and recently demonstrated capacity for efficient and specific translation, circRNAs have emerged as a promising alternative to linear mRNAs in vaccine development [48] (Fig. 3). Recent studies have successfully generated artificial circRNAs capable of robust protein expression,

including constructs that elicit antiviral immune responses [180-182], and used them in the development of prototype vaccines against SARS-CoV-2 [182]. Notably, circRNAs have been shown to sustain protein expression for longer periods compared to their linear mRNA counterparts. In particular, circRNA constructs encoding the VFLIP-X spike protein (modified VFLIP containing six amino acid substitutions) were developed. A vaccine prototype based on the circRNA-mediated expression of VFLIP-X induced neutralizing antibodies in mice that persisted for up to seven weeks following booster immunization, demonstrating efficacy against SARS-CoV-2 and its variants [182].

At the same time, circRNAs themselves can modulate immune system activity [86, 227]. They are capable of activating the innate immune sensor RIG-I and, under certain conditions, may even trigger autoimmune responses. The immunostimulatory potential of circRNAs depends on factors such as their biogenesis, structural features, and method of production. Thus, circRNAs of different origins can elicit distinct cellular immune responses. RNA-binding proteins play a crucial role in this process by recognizing specific secondary structure motifs within circRNAs and mediating their discrimination as “self” or “non-self.” Therefore, when developing safe circRNA-based vaccines, it is essential to carefully consider the immunomodulatory properties of circRNAs and their potential contribution to the overall immune response.

Recently, the first data on circRNA-based oncolytic vaccines have appeared [228]. The authors reported that a vaccine incorporating the tumor-specific circRNA *circFAM53B* and its non-canonically encoded cryptic peptides induced a robust anti-tumor immune response in mouse models of melanoma and breast cancer [229]. In addition, a recent study described the development of a circRNA encoding chimeric antigen receptor (CAR) proteins, the key tools for the T cell-mediated tumor eradication. The authors demonstrated that the circRNA *CAR* suppressed tumor growth and reshaped the tumor microenvironment in mice [230].

Although circRNA-based vaccines have a potential to enhance therapeutic efficacy, several challenges remain unresolved. These include the synthesis of target circRNAs with minimal impurities, optimization of delivery systems, and mitigation of unwanted immune responses. Intensive research efforts are underway to address these issues, including the application of bioinformatics approaches and artificial intelligence-based methods [231-233]. Another important concern is the potential for off-target effects arising from the extensive interactions of circRNAs with bioactive molecules, such as nucleic acids and proteins. Growing evidence suggests that the development of safe and effective circRNA vaccines requires a comprehensive understanding of circRNA interaction networks and their regulatory roles at a genome-wide level.

CONCLUSION

In this review, we examined the key features of circRNAs, including their structure, biogenesis, functions, and practical applications. CircRNAs play diverse roles in the regulation of gene expression: they can act as molecular sponges for miRNAs and proteins, modulate alternative splicing and chromatin organization, regulate R-loop formation, and serve as templates for translation. Due to their high stability and tissue-specific expression patterns, circRNAs are regarded as promising biomarkers in the diagnostics of a wide range of diseases and in forensic applications. In addition, they can function as therapeutic targets and even as standalone therapeutic agents. Particular attention is given to their potential in the diagnostics and treatment of neurological disorders, including neurodegenerative diseases and ischemic stroke. The use of circRNAs in genome editing technologies to enhance the efficiency of CRISPR–Cas systems is very promising. Furthermore, the development of next-generation vaccines based on circRNAs, including antiviral and oncolytic vaccines with improved stability and immunogenicity, represents a rapidly evolving field.

Despite substantial advances in circRNA research, many important questions remain unresolved. CircRNAs are still sometimes regarded as byproducts of aberrant splicing – molecules that exist in cells but lack functional significance. Moreover, although numerous circRNAs and their potential roles have been predicted using bioinformatic approaches, many of these predictions have not yet been validated experimentally, increasing the risk of overinterpretation or mischaracterization of their biological relevance. Key challenges include elucidating circRNA-centered regulatory networks, particularly their roles in modulating low-affinity molecular interactions; developing reliable methods for the preparative isolation of pure circRNAs; overcoming obstacles related to their efficient and targeted delivery *in vivo*; and addressing a potential ethnic specificity of circRNA-based therapeutic strategies. CircRNA research remains a rapidly evolving field, significant not only for advancing fundamental biological knowledge but also for enabling the development of innovative technologies in modern medicine and biotechnology.

Abbreviations

circRNA	circular RNA
EIciRNA	exon–intron circular RNA

IRES	internal ribosome entry site
mecciRNA	mitochondrial-encoded circular RNA
miRNA	microRNA
mRNA	messenger RNA
cp-circRNA	chloroplast genome-encoded circRNA
TDMD	target RNA-directed miRNA degradation
tricRNA	tRNA intronic circular RNA

Contributions

I.B.F., O.Yu.S., S.A.L., and L.V.D. developed the concept and supervised the study; I.B.F., I.V.M., and E.V.Ts. wrote and edited the manuscript; I.B.F. and E.V.Ts. prepared the figures; S.A.L. and I.B.F. acquisition funding.

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Ethics approval and consent to participate

This work does not contain any studies involving human or animal subjects.

Conflict of interest

The authors of this work declare that they have no conflicts of interest.

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