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Circular RNA: the rings of power over cell

V. V. Gordiyuk, O. S. Mankovska

Institute of Molecular Biology and Genetics, NAS of Ukraine

150, Akademika Zabolotnoho Str., Kyiv, Ukraine, 03143

vasilij_gordiyuk@yahoo.com

Circular RNAs (circRNAs) are endogenous covalently closed single-stranded noncoding RNAs (ncRNAs) without the 5'-cap and 3'-poly(A) tail derived from alternative mRNA splicing. CircRNAs are expressed across all branches of evolutionary tree, including viruses and prokaryotes, and are evolutionary conserved. CircRNAs have a constitutive biological role. In this review, we summarized the latest data on circRNAs and highlighted their most important and studied functions in different biological processes.

Keywords: Circular RNA (circRNA), RNA binding proteins (RBPs), backsplicing model, lariat model, the circulating cell-free RNA, cancer-related circRNA

Introduction

The immense world of non-coding RNAs (ncRNAs) is full of surprises for researchers. In previous studies, scientists usually focused on the linear ncRNAs, such as long non-coding RNAs (lncRNAs) and micro-RNAs (miRNAs), especially after it was shown, that these linear ncRNAs have multiple functions in physiological and pathological processes [1, 2]. However, in the last decade circular non-coding RNAs attracted the attention of researchers due to the discovery of their unprecedented abundance and great diversity of forms and functions.

The circular RNAs (circRNAs) are considered as a class of endogenous covalently

closed single-stranded non-coding RNAs without the 5'-cap and 3'-poly(A) tail, which are derived from alternative mRNA splicing. The circRNAs are ubiquitous and play the constitutive and different biological roles [3, 4].

The first covalently closed RNA circles were discovered in the potato spindle tuber viroid in 1967 [5]. These circRNA-containing viroids are quite small (220–400 nucleotides) and are specialized exclusively on infecting the plant cells, replicating autonomously within the nucleus or chloroplasts of their host cells. Their genome forms the self-complementary base-paired rod-like structures [6]. In 1976, the circRNA was found in the Sendai virus [7].

The decade later, in 1986, Hepatitis delta (δ) virus (HDV) was considered to contain circRNA-encoded genome [8]. HDV is a satellite virus of the human Hepatitis B Virus (HBV) and the HBV envelope proteins (HBsAg) are necessary for it to form virus particles. The Hepatitis Delta virion was shown to be circular, single-stranded, negative sense RNA, composed of approximately 1700 bp [9]. HDV genome encodes a single open reading frame, which is translated to regulate viral replication [10].

In 1988, the circRNAs were described as products or intermediates of rRNA maturation in prokaryotes [11]. In thermophilic archaea their circular, protein-bound structure could evolve as protection from heat denaturation [12, 13]. Except viruses and prokaryotes, the circRNAs are also expressed across all branches of the eukaryotic tree of life.

The proper eukaryotic circRNAs were first detected by electron microscopy in cytoplasm of the human HeLa cells in 1979 by scientists at the Rockefeller University [14]. Later on, it was shown, that circRNAs are also present in protists [15, 16] and fungi, such as yeast [17]. Furthermore, circRNAs were described as intermediates of tRNA maturation in unicellular red algae [18]. In 1991, the first endogenous circRNAs, which are expressed in higher eukaryotes, were identified [19]. In 1993, it was also demonstrated, that circRNAs may rise by the splicing error of a human *ets-1* proto-oncogene [20]. In 2012, it was shown that circRNAs can be the transcriptional products of thousands of human genes and in hundreds of cases constituted the dominant RNA isoform [21].

The classification and characteristics of circRNAs

Generally, expression of 20 % of eukaryotic genes, which are currently active in a cell, is associated with circRNA production [22, 23].

Today the circRNAs can be generally divided into eight categories according to their origin: exonic circRNAs (ecircRNA) [24], circular RNAs from introns (ciRNAs) [25], exon-intron circRNAs (EiciRNA) [26], intergenic circRNAs [27], interior circRNAs (i-circRNAs) [28], antisense circRNAs (as-circRNAs) [4], tRNA intronic circRNA (tricRNAs) [29], and fusion circRNAs (f-circRNAs) [30] (see table 1 for details).

EcircRNAs are accounting for approximately 85 % of all types of circRNAs [31]. Up to six exons could be spliced in inverted order within one circRNA [32]. The exonic circRNA molecules range from 100 to over 4000 nucleotides; the size of intronic circRNAs varies from 200 to over 3000 nucleotides [33]. Majority of exonic circRNAs are often located in the cytoplasm, while a small part of circRNAs, consisting of introns, could be found in the nucleus [24].

CircRNAs are extremely abundant and possess great diversity: more than 20000 different circRNAs were identified in eukaryotes by high-throughput sequencing and novel computational approaches for non-polyadenylated RNA transcripts [24]. The fluorescent *in situ* hybridization (FISH/ISH) techniques can be also used for detection of the circRNA transcript with a DNA oligo probe in the cells or tissue samples [34].

CircRNAs are more stable than their cognate linear mRNAs. The average lifetime of 3'→5'-linked circRNAs is 19–24 h, however,

it can be up to 48 h [35]. This is 2–5 fold longer (in certain cases - up to 10-fold) compared to the linear mRNAs, that have the average lifetime of 4–9 h [36].

On a genome-wide level, usually 3–10 circRNA isoforms are produced per gene [37]. CircRNAs are usually expressed at low levels, compared with messenger RNAs (mRNAs). The vast majority (>90 %) of circRNAs are present as just 1–10 molecules per cell [38]. The length of the mature circRNA dictates the mode of nuclear export [39].

CircRNAs are usually expressed in cell-specific and tissue-specific manners [40]. In the study of nematode embryogenesis several circRNAs, present in oocytes, were not expressed later in 1- or 2-cell embryos, which is the evidence of their potential role in developmental processes [41]. In adult, non-pathological (normal) tissues expression of circRNAs is characterized by conservation and complexity [42]. CircRNAs are evolutionarily conserved at the sequence level among different species [43].

A number of research works were concentrated on explaining the mechanism of transcript circularization, especially on characterization of genomic sequences which can generate circular transcripts. One of the observations, that explained the mechanism of circularization, was the link between repetitive elements and circular RNA biogenesis. This link was first described in the mouse *Sry* locus, which produced circRNA when it was flanked by repetitive complementary sequences, and lost this ability when repeats were completely removed [44], later such phenomenon was discovered for human and plant circRNAs.

The most widespread repetitive elements in human genome are Alu repeats. By the means

of computational analysis, it was found that human exons that encode circRNAs are flanked by introns enriched in Alu elements, and approximately 90 % of human circRNAs have complementary Alu elements in their flanking introns. Therefore, the model of possible circularization was suggested, according to which the intronic repeats may base pair to one another, bringing the splice sites into close proximity [43].

The participation of repetitive elements in regulation of transcript formation can also appear in active manner, due to the fact, that some of these repeats continue to retrotranspose (for instance, LINE 1 and Alu). Thus, the new retrotransposition could switch gene transcription from a protein-coding mRNA to a circular RNA and alter gene function. Such evidence can come as explanation of a number of diseases, associated with retrotransposition insertions – even if it does not disrupt gene structure, it can deregulate its function [45, 46].

One more way of transposone-associated circRNA biogenesis is connected with their ribozyme activity. The new family of genome-encoded circRNAs (self-cleaving or hammerhead ribozymes) was found in plants, and authors described them as small and nonautonomous retrotransposable elements. These ribozymes are spread in plant genomes through small non-coding circRNAs [47].

The existence of circRNAs was demonstrated in blood cells and in cell-free compartments. CircRNAs are significantly enriched in platelets: 17-188-fold, compared with the nucleated tissues [48], that's why they are high in human peripheral whole blood [49]. CircRNAs were also detected in bone marrow cells [50]. More than 1000 circRNAs were

identified in human serum exosomes. Exosomal circRNAs can participate in cell-to-cell communication as a messenger to mediate multiple signaling pathways [51].

Turnover of circRNAs

Biogenesis of circRNAs

Various types of circRNAs are generated in different ways. Up to date, it is known, that more than 95 % of human genes are alternatively spliced [52]. According to existing research data, namely alternative splicing is the main mechanism of circRNA formation. The discovery of inverted, out-of-order spliced exons in ssRNA, referred to as “scrambled exons” in humans [19], gave the first evidence, that these molecules might originate from the protein-coding open reading frames (ORF). However, introns, located in the ORF of a protein-coding gene, also can circularize [53].

In a case of exon-originated circRNAs there were proposed three models of their possible formation: the lariat model [54], backsplicing model and RNA-binding protein (RBP)-driven circularization [55] (Fig. 1).

The model of lariat-driven circularization is based on concept that the exon is skipped along with RNA folding, and exonic circRNAs or EIciRNAs are formed, depending on the removing or keeping introns. When a pre-mRNA is spliced, the 3-hydroxyl of the upstream exon interacts with the 5'-phosphate of the downstream exon to form a covalent linkage, producing a lariat that contains exons and introns. Exon skipping results in production of the mature linear mRNA as well as the intron lariat containing exon. This lariat is re-spliced to generate the mature circRNA as well as a

double lariat structure that is subsequently debranched and degraded [54] (Fig. 1, A).

In intron-pairing-driven circularization (base-pairing-driven circularization), the formation of exonic circRNAs or EIciRNAs is regulated by base pairing in introns on both sides of pre-mRNA (Fig. 1, B). Base pairing between downstream introns and upstream introns (based on inverse-repeating or complementary sequences) results in back-splicing and production of the circRNA, containing the single or multiple exons [55, 24]. The introns are removed or retained to form ecircRNA or EIciRNA.

The last of existing exonic circRNA generation models proposes the involvement of RNA-binding proteins (RBPs) and following RBP-driven circularization. RBPs can bind to the specific sequence of introns, shorten the distance between the donor site and the receptor site, and initiate the biogenesis of ecircRNA [55] (Fig. 1, C).

Non-exonic circRNAs can be formed either from introns of one gene, or from non-exonic sequences between two different genes in a case of intergenic circRNA [27] (Fig. 1, D). In the formation of intronic circRNAs, the intron lariat is formed with the combination of conserved motifs, located upstream and downstream of introns (7-nt GU-rich element and 11-nt C-rich element to escape debranching and exonucleolytic degradation) [25] (Fig. 1, E). Interestingly, tRNA intronic circRNAs acquire their stable circular structure via pre-tRNA splicing. The introns, which were removed during this process, then circularize using back-splicing mechanism [29] (Fig. 1, F).

The biogenesis of circRNAs is also regulated by splicing factors. At the same time, these RNA molecules in some cases regulate

alternative splicing, which helps to maintain the balance between circRNA biogenesis and canonical splicing [56].

Export and possible way of degradation of circRNAs

CircRNAs are exported to the cytoplasm by RNA helicase URH49 or ATP-ase UAP56 [57, 58].

CircRNAs can be degraded by endonucleases. MicroRNA/RISC/AGO2-mediated cleavage could be a dominant degradation mechanism. For example, the circRNA produced from the CDR1as transcript is targeted for degradation by the miR-671, that guided the cleavage [59].

There is another possible pathway of circRNAs degradation, induced by selective context-dependent posttranscriptional meth-

ylation of adenosines in RNAs. The following recruitment of the YTHDF2 protein to N6-methyladenosine (m6A) relocates affected mRNAs to P-bodies for degradation [60].

Additionally, the transport by the extracellular vesicles might be also implicated in reduction of cellular circRNA content. Expulsion of circRNAs into the extracellular space by exosomes can be a way to clear and degrade circRNAs. Alternatively, exosome can protect circRNA from degradation [61].

The interactions between circRNAs and proteins

The interactions between circRNAs and proteins are mainly analyzed by the RNA pull-down assay or RNA immunoprecipitation (RIP). Also, studies of co-localization between circRNAs and proteins, using fluorescent mi-

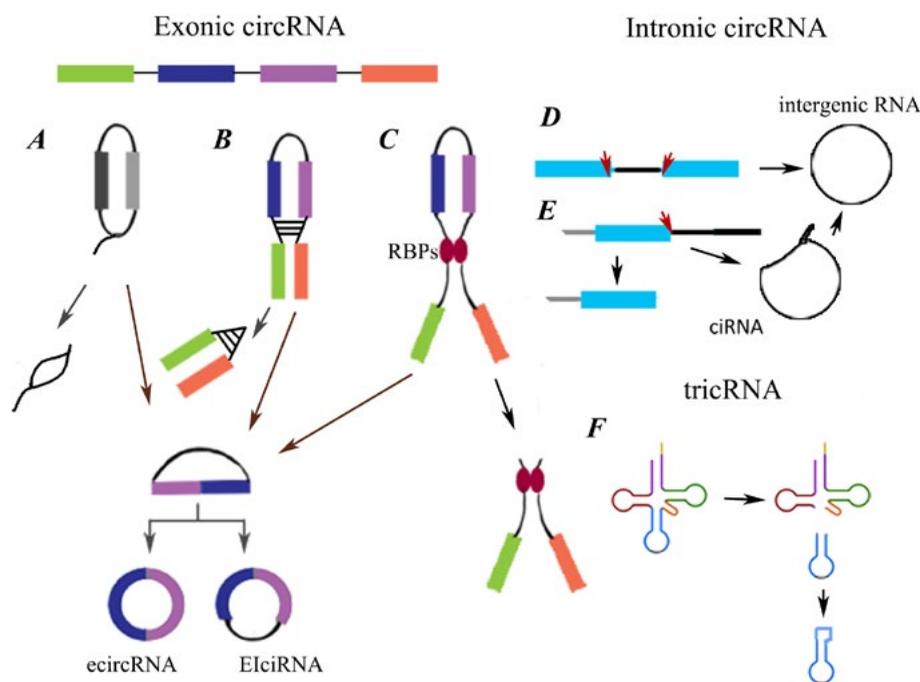


Fig. 1. Biogenesis of circRNAs. The ways of biogenesis of ecircRNAs and ElciRNAs: (A) – lariat-driven, (B) - by intron pairing, (C) – through binding RBPs (D) The biogenesis of intergenic circRNAs. (E) The biogenesis of ciRNAs. (F) The generation of tricRNA. Abbreviations: circRNAs - circular RNAs; ecircRNAs - exonic circRNAs; ElciRNAs - exon-intron circRNAs; ciRNAs - circular intronic RNAs; RBPs - RNA-binding proteins; tricRNA - tRNA intronic circular RNAs.

crosscopy, were performed, to demonstrate the close proximity of these molecules [62].

RBPs represent a broad class of proteins involved in gene transcription and translation; they contain different types of domain motifs [63]. RBPs regulate almost every aspect of the circRNA lifecycle: splicing, processing, folding, stabilization, localization and degradation [64].

Vice versa, circRNAs can regulate expression and function of RBPs. CircRNAs display a variety of functions in different tissues or at developmental stages, via binding with various functional proteins. CircRNAs may bind, store, sort, and sequester proteins to particular sub-cellular locations, and also act as dynamic scaffolding molecules, modulating protein-protein interactions [62].

The highly conserved RNA-editing enzyme ADAR (Adenosine deaminase acting on RNA) binds the double-stranded RNAs by targeting Alu repeats in human cells. ADAR1 antagonizes circRNA biogenesis through A-to-I editing of intron pairs flanking circularized exons, thus diminishing the complementarity and stability of these intron pairs' interactions [65]. DHX9, an abundant nuclear RNA helicase, has the unique domain organization, resembling ADAR. Silencing of DHX9 leads to increased circRNA production [66].

RNA binding protein FUS regulates circRNA formation by binding introns, flanking back-splicing junctions [67]. CircRNA production is further controlled by serine-arginine (SR) proteins [68]. For instance, heterogeneous nuclear ribonucleoprotein L (HNRNPL) regulates the alternative splicing of multiple RNAs and is involved in the regulation of circRNAs in prostate cancer [69]. Quaking (QKI), as a kind of alternative splicing factor, promotes

circRNA generation by binding to its intronic binding motifs [70]. RBPs are involved in transcriptional regulation of target genes and also in post-transcriptional regulation [71]. CircRNAs function as the miRNA sponges with the help of Ago, one of the RBPs [72]. CircRNAs compete with mRNA to combine with RBP, to influence the mRNA translation. These two kinds of molecules also regulate the specific modifications (DNA-methylation) [73]. Additionally, RBPs are involved in the potential extracellular transport pathway [64].

A dynamic circRNA-protein interaction.

CircRNA-protein interactions are dependent on the variety of the circRNA tertiary structures in various cell/tissue environments, resulting in higher protein binding capacity, than those of linear RNA sequences [62].

Endogenous cytosolic circRNA, a circular transcript from forkhead box O3 (circ-Foxo3) can facilitate, localize and regulate functions of the proteins. The major tertiary structure of circ-Foxo3 allows binding to the stress related proteins in cardiomyocytes and cardiac fibroblasts. Expression of circ-Foxo3 results in reduced nuclear translocation of transcription factors E2F1 and HIF- α and also in induction of cardiac senescence [74]. Moreover, this circ-Foxo3 structure is responsible for binding to Mdm2 (the E3 ubiquitin-protein ligase) and p53 in a breast cancer cell line. By binding with these proteins, circ-Foxo3 acts like protein scaffold and promotes poly-ubiquitination and degradation of p53 [62, 75]. Also, circ-Foxo3 controls its interaction with p21 and CDK1 in mouse fibroblast NIH3T3, via forming ternary complexes with p21 and CDK2 resulting in repression of the cell cycle entry [76].

Role of RBPs in circRNA cell-to-cell transmission

Various RBPs can be critical factors for the promotion of circRNA transmission in the parent cells [64]; they can serve as the intra-cellular inducers of circRNA, loaded in exosomes in the recipient cell. For example, two circRNAs, KIRKOS-73 and KIRKOS-71, are encoded by the *WWOX* parent tumor suppressor gene and transferred by exosomes, regulating p53 expression in the recipient cells [77]. So, parent cells may influence RBP expression in the recipient cells and regulate their circRNA biogenesis via exosome secretion [70]. RBPs may conceivably be necessary for compacting the relatively large nucleic acid structures into the small interior spaces of exosomes [78].

Functions of circRNAs

Accounting the multiplicity of the ways of biogenesis and origins of circRNAs it is not surprising, that their functions are also multiple and diverse. We divided them into several main categories. Yet, some of investigated functions of circRNAs are difficult to categorize, so we listed them separately.

1. Circular RNAs can be involved in regulation of gene expression through direct and indirect mechanisms. The most studied examples are described here:

a) CircRNAs can function to adsorb miRNAs (miRNA sponge) and regulate the target genes of those miRNAs by this mechanism. This model was proposed in 2013 [72]. The example is the circular transcript CDR1as (ciRS-7), which contains 66 selectively conserved miRNA target sites [4]. Moreover, several circRNAs possess microRNA response

elements (MREs), which enable them to interact with miRNAs to govern target gene expression [79].

b) At the same time, certain circRNAs could also serve as a miRNA reservoir, contributing to their stabilization [80].

c) Several ciRNAs and ElciRNAs can directly act as transcriptional regulators. The mechanism of action of intronic circRNAs in the nucleus suggests a new cis-regulatory role for circular transcripts in expression of their parental coding genes. It was shown, that some circRNAs, for example ci-ankrd52 and ci-sirt7, are associated with the PolII elongation complex and also specifically associated with phosphorylated PolII. In this way they can positively regulate Pol II transcription of their parent genes [25]. ElciRNAs, such as circEIF3J and circPAIP2, also regulate their parental genes by specific RNA-RNA interaction between U1 snRNA and ElciRNA. It results in formation of the complex between RNA polymerase II and U1 snRNP and in increased expression of the parental genes of ELciRNAs [26].

d) Additionally, circRNAs can regulate alternative splicing by binding to DNA [81]. CircMbl biogenesis competes with pre-mRNA splicing [56].

2. CircRNAs can bind to proteins and work as protein scaffolds and, moreover, regulate their localization [76]. The several examples of it are described here: circ-Foxo3, predominantly localized in the cytoplasm, interacts with ID1, E2F1, HIF1 α , and FAK, leading to their cytoplasm retention; circ-Amotl1 can promote the nuclear localization of c-Myc, STAT3, PDK1, and AKT1 by direct interactions with all of them; using its different regions, circ-Dnmt1 can bind to p53 and Auf1

and facilitate their nuclear localization through the interaction with HuR protein, circAGO2 facilitates translocation of HuR from the nucleus to the cytoplasm [75]. As single circRNAs can bind to multiple units of RBPs, they serve as stores of RBPs [82].

3. CircRNAs can regulate protein translation: high levels of circPABPN1 derived from PABPN1, repress HuR binding to PABPN1 mRNA through binding to HuR itself, causing a reduction in PABPN1 mRNA translation [71].

4. CircRNAs can encode peptides under certain conditions [83]. In eukaryotic cells, some circRNAs contain an Internal Ribosome Entry Site (IRES) for ribosomal 40S subunits, thus stimulating 5'Cap-independent initiation of translation [38]. Approximately 13 % of the total circRNAs show a N6-methyladenosine (m6A) modification, which drives translation initiation through recruitment of the initiation factor eIF4G2 [84]. These two alternative translation initiation processes of circRNAs are generally activated under cellular stress and promote expression of specific stress response genes [85]. Proteins, translated by circRNA, can act synergistically with the protein expression products of the parental gene and often function together. For example, FBXW7-185aa is encoded by circ-FBXW7 and regulates the stability of the c-Myc oncoprotein [86].

Except this, some circRNAs (described for circANRIL) can modulate the ribosomal RNA maturation [87]. CircRNAs also can regulate histone modifications. For instance, circ-MRPS35 acts as molecular scaffold for KAT7 histone acetyltransferase at the promoters of FOXO1 and FOXO3a and mediates acetylation of H4K5 in this region [88]. Additionally,

some circRNA can be reverse-transcribed *in vivo* into cDNA and integrated into the genome to generate a circRNA-derived pseudogene, playing an important role in cell differentiation and cancer progression [89].

CircRNAs as regulators of physiological and pathological processes

The circRNAs play various functions in the cell. They participate in neuronal development and are extraordinarily expressed in the mammalian brain due to a potential to regulate synaptic function [37]. A number of circRNAs can be involved in immune responses, which is described later in this article. A global accumulation of circRNAs during aging was found across different species [90]. CircRNAs can be critical factors for senescence and survival of the cell [91]. Moreover, circRNAs are involved in the age-related diseases, e.g. Alzheimer's disease [92, 28]. Also, many studies connected the presence of circRNAs with cardiovascular diseases [93], diabetes [94], and cancer [95].

Role of circRNAs in development

For today, the presence of circRNAs has been identified in a number of tissues: nervous tissue, heart and skeletal muscle, and other. Although their functions in different organs are only partially described, the role of their regulation is undoubtedly important, either for the maintenance of organ function and homeostasis or in the development of those tissues.

Analysing Guizhou miniature pig *S.scrofa* circRNAs on 0 (D0), 30 (D30) and 240 (D240) days of postnatal development, Liang and coauthors detected 1,489 circRNAs expressed in muscle tissue at different stages of development, but relatively small subset of them (101 and 83,

respectively) is differently expressed in the D0 versus D30 and D30 versus D240. The difference between D0 and D30 was represented by circRNAs, which can be associated with muscle development, whereas the comparison of the D30 and D240 groups shows that the host genes of differentially expressed circRNAs were associated with homeostatic processes such as ion homeostasis and cation homeostasis. The authors conclude that circRNAs may regulate the growth of muscle tissue during early post-natal muscle development via influence on cell proliferation and fusion [96].

For today, research works discovered functional features of a number of circRNAs, present in human muscles at different stages of development, giving direct or indirect evidence of their roles in myogenesis. Among them circ-ZNF609, circRBF2, circSVIL, circLMO7, circFUT10, circSNX29, circFGFR4, circFGFR2 and circHIPK3 act like miR sponges, but some of them may play additional role, e.g. circ-ZNF609, which contains the open reading frame, but those mechanisms stay unrevealed yet [97].

Circular isoform of NCX1 is a highly expressed and conserved circRNA. NCX1 is a sodium/calcium exchanger, responsible for transporting calcium out of the cardiomyocyte after contraction. Both qPCR and sequencing data demonstrated, that circular isoforms of NCX1 are induced in the fetal heart and during *in vitro* cardiomyocyte differentiation [98].

In comparison to other tissues, human brain is characterized by relatively high expression of circRNAs, presumably, due to preferential back-splicing [99]. Chen *et al.* study of circRNA from embryonic neocortex at early and late stages of its development showed, that

6550 circRNAs were expressed at both stages. Interestingly, 39 % of all expressed genes at the early and late stages also produced circular transcripts. They identified 104 differentially expressed genes across the early and late stages. The strongest differences were observed in expression of circular transcripts of SATB homeobox 2 (SATB2), regulating synaptic membrane exocytosis 1 (RIMS1), and CTC-525D6.1, which were significantly up-regulated at the late stage as compared to the early stage. At the same time, the linear forms of listed genes did not expose meaningful differences in their expression among the stages of embryonic brain development. All of these three genes are strongly and specifically devoted to brain tissue functioning and, thus, it was concluded, that the presence of their circular transcripts plays important, but still not fully understood, role in the human fetal brain development [100].

CircRNAs in immune responses

One of detected implications of circRNAs in immune responses is the suppression of viral replication and protection from bacterial infection through interaction with pattern recognition receptors (PRRs) – the main receptors of innate immunity. For instance, a recognition receptor RIG-I co-localizes with foreign circRNAs. Exogenous circRNAs can activate a RIG-I-induced cellular immune response pathway, which leads to suppression of viral replication. The mechanism, by which the circular RNA is made, is the key determinant of whether the transcript would be recognized as self or non-self. It predominantly depends on the self introns, which can, irrespective of the exonic sequence, program the circRNA iden-

tity by defining the set of RNA binding proteins, which mark RNA as “self” [101].

The immune factor NF90/NF110 can promote the circRNA formation by stabilizing the intron complementary sequence. DsRNA binding proteins NF90/NF110 regulate the expression of endogenous circRNAs [102]. Upon viral infection, NF90/NF110 is exported from the nucleus to the cytoplasm; it binds viral transcripts and suppresses viral replication [103]. NF90/NF110 shows the higher affinity to circRNAs, than linear RNAs [102].

Additionally, mcircRasGEF1B can help to protect cells from bacterial infection by enhancing the stability of mature ICAM-1 mRNA [104].

The mentioned ability of circRNAs to function as “microRNA sponges” is the mechanism, by which certain circRNAs are able to increase expression of target proteins, involved in antiviral responses or tumor immunity, such as PD-L1 [105].

CircRNAs also can be involved in differentiation of immune cells. The evidence of such ability is 189 circRNAs, which were found differentially expressed in M1 and M2 macrophages and, thus, can be involved in macrophage differentiation and polarization [106].

CircRNAs in cancer

Multi-functional cancer-associated circRNAs can possess both oncogene and tumor suppressor properties in tumors. CircRNAs influence the invasive capacity of tumor cells in various ways, including competition with miRNAs, translation into proteins, activity as miRNA reservoirs and formation of the fusion circRNAs [4,107]. CircRNAs can also bind to proteins or function as protein decoys in cancer cells. For

example, circ-Amot1 is able to bind and retain c-Myc in the nucleus, where it stabilizes c-Myc, up-regulates its target genes, and leads to increased cell proliferation, reduced apoptosis, and a highly tumorigenic phenotype [108].

Expression of circRNA is often associated with the late stage of tumors and poor prognosis. Exosomal circRNAs are also implicated in cancer drug resistance via not fully understood mechanisms. Presumably, they act activating such cellular pathways as WNT and promoting the cell to turn to the stem cell phenotype. As was demonstrated for CRC, exosomes can be transferred from drug-resistant to drug sensitive cell and modulate their chemo-resistance [109].

CircRNAs regulate the epithelial-to-mesenchymal cell transition (EMT). Of note, one of the key mechanisms, by which TWIST1 (the EMT-controlling transcription factor) promotes tumorigenesis, is regulating circRNA [110]. Production of one-third of EMT-related circRNAs was dynamically regulated by QKI, a key RBP in this process [70].

CircRNAs play important roles in cancer progression through modulation of many of the hallmarks of cancer [95]. For example, circFoxo3 induces apoptosis in cancer cell lines [79]. It was shown, that expression of this circular RNA was downregulated in tumors and cancer cell lines in comparison with normal tissues and non-cancerous cells. The mechanism of its action has dual nature and is based on modulation of the poly-ubiquitination function of MDM2: first, it can increase poly-ubiquitination of mutant p53, binding to both p53 and MDM2, and second, Foxo3 protein, which does not bind to circFoxo3, is freed from degradation, that leads to increased

Foxo3 activity, promoting Puma expression and cell apoptosis [75]. Noteworthy, the major tertiary structure models of circ-Foxo3 are different in cancerous and normal cells [62].

CircPRMT5, which acts as a sponge for mir30-c, was found frequently up-regulated in bladder urothelial carcinoma, moreover, its up-regulation was associated with lymph nodes

Table 1. The characteristics of different categories of circRNAs.

Name	Origin	Location	Sequence feature	Major function	Reference
ecRNA/ ecircRNA	exon	cytoplasm	Formed by cyclization of exons containing the reverse complementary sequence of introns and selective cyclisation; joint site: 3'-5' phosphodiester bond	miRNA or RBP sponge; regulation translation	Zhang X. <i>et al</i> , 2014. [24]
EIciRNA	exon and intron	nucleus cytoplasm	Formed by cyclization of exons containing the reverse complementary sequence of introns and selective cyclisation; joint site: 3'-5' phosphodiester bond	Enhance transcription of the parental genes	Li Z. <i>et al</i> , 2017 [26]
ciRNA	intron	nucleus cytoplasm	7-nt GU-rich element close to the 5-splice site and an 11-nt C-rich element near the branch point; joint site: 2'-5' phosphodiester bond	Regulation oRNA pol II transcription; Regulation transcription of parental genes	Zhang Y. <i>et al</i> , 2013 [25]
intergenic circRNA	intronic circRNA fragment	NA	Formed by two intronic circRNA fragments (ICFs) flanked by GT-AG splicing signals acting as the splice donor (SD) and splice acceptor (SA) of the circular junction.	NA	Gao Y. <i>et al</i> , 2015 [27]
interior circRNA/ i-circRNAs	interior regions of exon, intron, and intergenic transcript	NA	Back fusion points associated with motif AC/CT	Disgulation in Alzheimer's Disease	Liu X. <i>et al</i> , 2020 [28]
Antisense circRNA	exon, that transcribe from the opposite strand	cytoplasm	Cyclisation of exons	miRNA sponge; ribosomal RNA maturation	Memczak S. <i>et al</i> , 2013 [4]
TricRNA	intron	NA	Splicing pre-tRNA intron an the bulge-helix-bulge (BHB) splicing motif by tRNA splicing endonuclease (TSEN); joint site: 3'-5' phosphodiester bond	NA	Lu Z. <i>et al</i> , 2015 [29]
Fusion circRNA	exons of distinct genes	NA	Produced from transcribed exons of distinct genes affected by aberrant chromosomal translocations	Disgulation in cancer	Guarmerio J. <i>et al</i> , 2016 [30]

Note: NA – no available

invasion, metastasis and poor survival prognosis. Due to the absence of significant up-regulation of circPRMT5 in several other studied cancers, it was supposed, that high level of this circular transcript may be specific for bladder cancer. It was shown, that circPRMT5 can be found in serum and urine exosomes from bladder cancer patients and its high level here is associated with metastasis and poor prognosis. Due to those data, circPRMT5 was proposed as potential novel target for anticancer therapy [111].

Zhang *et al.* identified the 87-amino-acid peptide, encoded by the circular form of the long intergenic non-protein-coding RNA p53-induced transcript (LINC-PINT). The expression of this peptide and its corresponding circRNA was decreased in glioblastoma compared to normal tissues. The authors demonstrated the ability of this transcript to suppress glioblastoma cells' proliferation *in vitro* and *in vivo* by direct interaction with polymerase associated factor complex (PAF1c) following the inhibition of numerous oncogenes' transcription [112].

The circular RNAs derived from viruses play exceptional role in cancer development. In 2018 Tuna Toptan *et al.* claimed the discovery of circRNAs in Epstein-Barr virus (EBV) and Kaposi's sarcoma herpesvirus (KSHV). Their experiments demonstrated, that either EBV or KSHV both encodes circRNAs, which are stably expressed in tumors, associated with these viruses. EBV abundantly expressed both exon-only and exon-intron circRNAs from the BamHI A rightward transcript (BART) locus (circBARTs). KSHV polyadenylated nuclear (PAN) RNA locus generated a swarm (>100) of multiply backspliced, low-abundance RNase

R-resistant circRNAs originating in both sense and antisense directions [113].

At the same year Ungerleider with coauthors published their article about EBV circRNAome. Using cell lines models of types I, II and III EBV latency in B-cell lymphomas and gastric cancers, they discovered, that EBV expresses numerous circRNAs, in both latency and reactivation. For instance, circRPMS1_E4_E3a and circRPMS1_E4_E2 are expressed in latency and have been detected in virus-associated patient tumor samples. Moreover, the majority of viral circRNAs, identified in their study, had the expression level similar or even higher, compared to cellular circRNAs. Also authors suggest, that the stress of transcription machinery during viral reactivation leads to increasing incidence of backsplicing compared to canonical splicing, thus, resulting in abundance of viral circular transcripts [114]. All these data place the viral circular RNAs as the potential biomarkers of virus-associated cancers.

It is no doubt, that cancer-related circRNAs and peptides, encoded by circRNAs, show a great potential as diagnostic markers, prognostic indicators and therapeutic targets for cancer treatment [115,116]. The comprehensive detection of different circRNAs and traditional markers may result in the higher diagnostic efficiency [117]. Nevertheless, the further investigations are needed to understand their mechanisms of action and the role in carcinogenesis.

Conclusions and Perspectives

Summarizing all information given above, circRNAs function as regulators of the pivotal cellular processes, e.g. transcription and trans-

lation; they can act alone, together with proteins and even be translated to peptides themselves. Thus, we can conclude, that the non-coding RNA community now is supplemented with new powerful member, which demonstrates amazing and diverse potential and functions.

Circular RNAs, although discovered several decades ago, stayed in the shadow for a lot of time, and only the research of 2010's uncovered their emerging capabilities. Participation in numerous cellular pathways and engagement in various physiological processes put them in a row with other non-coding RNAs, such as microRNAs, piwiRNAs, lncRNAs and others. Being the players in the development and aging processes, pathological conditions, such as cancer and other diseases, circular RNAs can serve as potential markers and, maybe, even therapy targets. Moreover, their presence in exosomes makes them probable markers for non-invasive diagnostics.

Nevertheless, a lot of questions (including the nuances of circular RNA biogenesis, the functions of different types of circRNAs, their interactions with microRNAs, proteins and other biomolecules) remain unrevealed yet. Therefore, further investigation is needed for better understanding of these RNAs to comprise the full picture of their role in living organisms.

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Кільцеві РНК – «кільця влади» над клітиною

В. В. Гордіюк, О. С. Маньковська

Кільцеві РНК (circRNA) є ендогенними ковалентнозамкненими одноланцюговими некодуєчими РНК (ncRNA), що не мають 5'-КЭПа і 3'-поліаденілювання, які утворюються внаслідок різних варіацій альтернативного сплайсингу мРНК. Експресію кільцевих РНК було виявлено у представників усіх груп еволюційного дерева життя, включаючи віруси та прокаріоти. Ці молекули є еволюційно консервативними. Кільцеві РНК відіграють постійну і універсальну біологічну роль. У даному огляді ми узагальнили останню наявну інформацію про

кільцеві РНК та виділили їх найважливіші та найкраще вивчені функції в різних біологічних процесах.

Ключові слова: Кільцеві РНК (circRNA), РНК-зв'язувальні білки (RBPs), модель бэксплайсинга, модель лассо, циркулююча позаклітинна РНК, circRNA, асоційовані з раком.

Кольцевые РНК – «кольца власти» над клеткой

В. В. Гордиук, О. С. Маньковская

Кольцевые РНК (circRNA) являются эндогенными ковалентно замкнутыми одноцепочечными некодирующими РНК (ncRNA), не имеющими 5'-КЭПа и 3'-полиаденилирования, которые образуются в результате различных вариаций альтернативного сплайсинга

мРНК. Экспрессию кольцевых РНК было обнаружено у представителей всех групп эволюционного древа жизни, включая вирусы и прокариоты. Эти молекулы являются эволюционно консервативными. Кольцевые РНК играют постоянную и универсальную биологическую роль. В данном обзоре мы обобщили последнюю имеющуюся информацию о кольцевых РНК и выделили их важнейшие и наиболее изученные функции в различных биологических процессах.

Ключевые слова: кольцевые РНК, РНК-связывающие белки (RBPs), модель бэксплайсинга, модель лассо, циркулирующая внеклеточная РНК, circRNA, ассоциированные с раком.

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