CircRNA: as a disease marker potential and research strategy

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Circular RNA (CircRNA) is an endogenous noncoding RNA with covalently closed cyclic structure. It is divided into exonic circRNA, intronic circRNA and exon-intron circRNA, based on their components. CircRNAs are well conserved in sequence and abundantly expressed in a tissue specific manner. They have a high stability due to resistance to exonuclease. Depends on their sequence, they perform many biological function including microRNA sponging activity, modulation of alternative splicing or transcription, interaction with RNA binding proteins, rolling translation and derivative of pseudogenes. They are involved in the development of a variety pathological condition including cardiovascular disease, diabetes, neurological diseases and cancer. Emerging evidences show that circRNA are likely to be potential targets for new clinical diagnostic markers or treatment of many diseases. In this review, we have described the potential relationship between circRNA and disease progression, methods and databases of cyclic RNA.

1 **CircRNA: as a disease marker potential and research strategy**

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34 based on their components. CircRNAs are well conserved in sequence and abundantly
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41 are likely to be potential targets for new clinical diagnostic markers or treatment of many
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43 disease progression, methods and databases of cyclic RNA.

44 **Keywords:** CircRNA; Diseases; Research strategy; Database
45

1. Introduction

47   Circular RNA (circRNA) was considered a class of endogenous non-coding RNAs(ncRNA) [1],
48 but now it has been confirmed that circRNA can be translated as a functional polypeptides to
49 function [2-5]. CircRNA was first discovered as genomes for certain RNA viruses in 1970s and
50 1980s. Unlike linear ncRNAs, CircRNA are formed with different combination of sequences and
51 domains, and based on that they can be divided into three categories: Exonic circRNA
52 (ecRNA)[6], Circular intronic (ciRNA)[7] and Exon—intron circRNA (ElciRNA)[8](Table 1)(Fig. 1).
53 Like other ncRNAs, the sequence and structure of circRNA determines its biological functions.
54 CircRNA are mainly found in the cytoplasm and they are highly stable compared to other
55 ncRNAs [9] . They are abundantly expressed and evolutionarily conserved across the eukaryotic
56 organisms [10,11]. CircRNA play very important role in many diseases including nervous system
57 disorders, cardiovascular diseases, diabetes and cancer [12,13]. CircRNA have special features
58 in biological system and their functions are tissue specific. CircRNAs govern gene expression
59 through guiding a number of other molecules, such as splicing factors, RNA polymerase II[14],
60 nuclear small ribose nucleoprotein (snRNP) [8,15] and miRNAs [16]. These interactions can
61 promote or inhibit the transcription of the corresponding mRNA.
62

Survey Methodology

64 Analysis : Through extensive reading of the literature, the role of circRNA in diseases and
65 research methods were analyzed, indicating the importance of circRNA and its research
66 prospects: including the author's own previous research results combined with others' research,
67 comprehensive analysis of the role of circRNA.

Subheadings

69 Disease marker potential; Research strategy

2. Biogenesis of CircRNA

CircRNAs do not have terminal structures such as 5' end cap and 3' end poly (A) tail, which are covalently closed to form a circular structure [14]. Jeck and his colleagues have proposed two different models of exon circularization. One is intron-pairing-driven circularization (Fig. 2a) and another model is lariat-driven circularization (Fig. 2b) [14]. The former is formed by the cyclization of ends of exons due to the complementary sequences of the introns. The matching between the reverse complementary sequences of introns leads to spatially closer and backward shear ring formation, which subsequently forms intronic circRNA by pairing the splice donor and acceptor sites. Typically, many lasso structures are formed by introns, but they are cleared by the branching enzyme degradation [17]; In lariat-driven circularization, heterogenous RNA (hnRNA) take parts in the process of RNA folding that brings exons closer to neighbouring exons and jump exons (Exon skipping), which results in generation of lasso intermediates and further splicing producing a mature exonic circRNA (ecRNA). Interestingly, all exons cannot form a circRNA, only the exons with reverse complementary Alu elements on both sides can pair and circularize, but it is not specifically required if there are other inverted repeats exist in the sequence [18]. DHX9 is a RNA helicase, which specifically binds to reverse Alu elements to guide the formation of cyclic RNA [19]. In addition, if the size of flanking introns is greater than that of exons, it favours easier cyclization [20]. Many proteins are involved in cricRNA biogenesis. The double-stranded RNA-binding domain containing immune factors NF90/NF110 are key molecules in circRNA biogenesis. NF90/NF110 promotes circRNA production in the nucleus by associating with intronic RNA pairs juxtaposing the circRNA-forming exon(s) [21]. HNRNPL promotes circular RNA formation via back splicing [22]. The RNA binding proteins such as MBL (muscleblind) [23], QKI (RNA-binding protein quaking I) [24] and FUS [25] also participate in the back-splicing process and cyclization of RNA. Interestingly, MBL promotes its own mRNA cyclization due to enrichment of putative MBL binding sites in flanking introns by which it connecting the flanking introns and sustaining cyclic structure and thus it promotes the exon cyclization. The monomeric QKI binds to both ends of intron flanking sites and they combined to form cyclic exons by bringing the two cyclic shear sites very close. FUS regulates circRNA biogenesis by binding the introns flanking the back-splicing junctions and this control can be reproduced with artificial constructs [25]. In contrast, the RNA editing enzyme ADARs (Adenosine deaminases acting on RNA) block circRNA formation by binds to complementary double-stranded area of flanking introns and abolishing the interaction of double stranded chains [26]. A recent study found that inhibition or slowing of pre-mRNA processing machineries such as spliceosomes leads to profound increase of circRNA production and this also enables the extend of read through s to downstream genes and production of circRNA [27].

3. Properties of CircRNA

CircRNA have several unique features and properties when compared with other linear RNAs as well as ncRNAs. A vast majority of them reside in the cytoplasm [29] and most of them are generated from exons, while few others come from introns or intron fragments. Several circRNA possesses microRNA response element (MRE), which enable them to interact with miRNAs and thereby they governs the target gene expressions [18,30]; Many circRNAs are

112 derived from pre-mRNAs and they regulate their own gene expression predominantly at post-
113 transcription level [31] and only few of them regulate transcription [7] Generally, circRNA show
114 tissue-specific and/or developmental-stage-specific expression pattern similar to that of
115 corresponding linear mRNA targets, and their expression level is >10 times higher than of the
116 linear mRNA [14] [32]. CircRNAs exist and detectable in many types of extracellular body fluids,
117 such as saliva, blood, and urine [33] [14]. More than 400 circRNA have been found in human
118 cell-free saliva (CFS) from healthy individuals [34]. CircRNAs show evolutionarily conserved
119 sequence features across different species [35]. The covalently closed loop structures with lack
120 of 5'-3' polarity and without polyadenylated tail fovours resistance to RNA exonuclease
121 degradation [36]. CircRNAs play diverse biological roles due to the fact that average half-life of
122 circRNA in most species is much longer than its linear counterpart [32,34].

123 **4. Function of CircRNA**

124 circRNAs have a variety of functions including miRNA sponging activity, modulation of
125 alternative splicing or transcription, regulating the expression of parental genes, interaction
126 with RBPs and alter their activity, rolling circle translation and generate pseudogenes.

127 *4. 1 CricRNA as MiRNA Sponges*

128 CircRNAs act as a competing endogenous RNAs (ceRNA) that contain shared miRNA
129 response elements (MRE) by which they sequestering miRNAs and preventing their interactions
130 with target mRNAs (Fig. 3a). The systematically validated circRNAs such as circRNA such as ciRS-
131 7 (CDR1as) [18,32] and Sry circRNA(circSry) [18,37] are produced from the mRNAs of cerebellar
132 degeneration associated protein 1 (CDR1) and dysregulated rat testis SRY respectively. During
133 embryonic developmental process in zebrafish, the expression of CDRlas reduces the brain
134 volume and its development is hampered. However, the exogenous delivery of miR-7 can bring
135 the brain volume and development to normal, which illustrate that CDRlas blocks miR-7 by
136 sponging functions [18,38]. New research finds that CDR1as sequence is overlapping the
137 sequence of lncRNA LINC00632 [39]. Another abundant circRNA derived from Exon2 of the
138 HIPK3 gene (circHIPK3) promotes cell proliferation by sponging miR-124. Interestingly, the
139 silencing of circHIPK3 but not HIPK3 mRNA inhibits cell growth. The luciferase screening assay
140 found that circHIPK3 possesses 18 potential binding sites to sponge 9 miRNAs. It can directly
141 bind to miR-124 and inhibits its activity [40]. In generally, there are only few circRNAs
142 containing enough miRNA-binding sites to function as a strong sponge and other circRNA are
143 exceptional cases [43, 44]. Another study found that knockdown of circHIPK2 expression
144 significantly inhibited astrocyte activation via the regulation of autophagy and endoplasmic
145 reticulum (ER) stress through the targeting of MIR124-2HG and SIGMAR1 [41]. CircHECW2 plays
146 a role in the epithelial-mesenchymal transition (EMT) pathway by competitively inhibiting miR-
147 30D, releasing ATG5, and thereby promoting the Notch1 signaling pathway [42].

148 *4. 2 CircRNA Modulates Alternative Splicing or Transcription*

149 CircRNAs participate in the regulation of alternative splicing and transcription, and thereby
150 they control gene expression (Fig. 3b). For example, circMbl is generated from the second exon
151 of the splicing factor MBL, which competes with canonical premRNA splicing. circMbl and its

152 flanking introns have conserved MBL binding sites that can strongly bind with MBL.
153 Interestingly, the alteration of MBL level significantly affects circMbl formation and this effect
154 depends on MBL binding sites in the flanking intronic sequences [23]. Studies have shown that
155 some circRNA are abundantly found in the nucleus and they regulate transcriptional activity by
156 interaction with polymerase II and homeopathic reaction. For instance, EIciRNAs interact with
157 small nucleonucleo proteins (snRNPs) to regulate the transcription of parental genes in a
158 homeopathic manner [43]. Li et al. found that cir-ITCH interacts with miR-7, miR-17 and miR-
159 214 and up-regulates the expression of ITCH [16]. During embryogenesis, sisR-4 promotes
160 transcription of its host gene by activating an enhancer present in the intron where sisR-4 is
161 encoded，which is essential for development [44]. HNRNPL directly regulates the alternative
162 splicing of RNAs, including encoding the androgen receptor, the key lineage-specific prostate
163 cancer oncogene [22].

164 *4. 3 CircRNA Interacts with RBPs*

165 Apart from miRNA regulation, circRNA can sequester RBPs and thus they control the
166 intracellular localisation and transport of RBPs and its associated mRNAs [45,46](Fig. 3c). Some
167 circRNA combine with RBP and ribonucleoprotein complex and prevent their activity, however,
168 circRNA functions as stores of RBP and ribonucleoprotein complex. EcRNA acts as a scaffold by
169 specifically binding with some protein molecules and providing interaction platform for RNA
170 binding protein, RNA and DNA. For example, CDRlas combines with the miRNA effector protein,
171 Ago2, and contributes to proteolysis function. Chen et al. found that there are cellular
172 differential mechanisms in the recognition of internal and external circRNA. They found that the
173 external circRNA induces activation of RIG-I-mediated cellular autoimmune effector pathways,
174 while endogenous circRNA does not induce this pathway due to binding of RBPs [47].

175 *4. 4 Rolling Circle Translation*

176 In eukaryotic cells, the cyclic mRNA can be translated by typical translation machineries
177 due to the fact that it contains an IRES (internal ribosome entry site) sequence and it can bind
178 directly to the ribosome (Fig. 3d). In prokaryotic cells such as E. coli, the circRNA contains a well
179 conserved ORF (infinite open reading frame) system, which enables the translation of
180 circularised RNA [48]. In eukaryotic system, the some circRNAs have binding sites for ribosomal
181 40S subunits, thus it can initiate the translation. This was proved in both in vivo and in vitro
182 studies. In E. coli system, the circRNA with an insertion of GFP (green fluorescent protein) in
183 open reading frame can successfully translate GFP [49]. Interestingly, circRNA also drive protein
184 translation by methylation of adenosine N6 (m6A) [2]. The protein translated by circRNA can
185 act synergistically with the protein expression products of the parent gene and they can
186 function together. For example, circ-FBXW7 can translate a new protein that inhibits glioma [3].
187 Circ-ZNF609 can directly translate proteins that participate in muscle formation [5]. In
188 prokaryotic cells, the proteins are generated from circRNA by means of rolling circle
189 amplification (RCA) analogous to polymerase reaction in the eukaryotic translation system,
190 which reveals that there is no need for multiple binding of translational machinery to the RNA
191 template. The circular amplification not only produces long and repetitive peptide sequences,
192 but also increases the productivity of the linear counterpart [50].

193 *4. 5 Generate Pseudogenes*

194   Studies have shown that stable circulatory molecules can be reversed transcribed and
195 integrated into the genome to form a circRNA-derived pseudogenes [51] (Fig. 3e). The
196 bioinformatic analysis of mouse genome using computational pipeline (CIRCpseudo) found that
197 at least 33 pseudogenes are possibly derived from the same circular RNA at the RFWD2 (ring
198 finger and WD repeat domain 2) locus (circRFWD2) and nine of those pseudogenes are from
199 exons (exons 2 to 4 or 5) of circRFWD2. It is well documented that pseudogenes play an
200 important role in the cell differentiation as well as in cancer progression [52].

201 **5. CircRNA in disease development and progression**

202   The best known circRNA such as ciRS-7 and CDR1as, the inhibitors of miR-7 microRNA, are
203 critical ncRNA known to be involved in various diseases including, cancer, neurodegenerative
204 diseases, diabetes, and atherosclerosis. Thus, the complex association of circRNA with critical
205 microRNAs and other gene families, circRNA might have important role in the development and
206 progression of various diseases (Table 2).

207 **6 Methods of CircRNA detection and characterisation**

208 *6. 1 Preliminary Purification and Identification*

209 6. 1. 1 Molecular biology method

210   The loop structure of CircRNA has high stability compared to Linear RNA and it is resistance
211 to enzyme digestion. Therefore, enzymatic digestion method can be used for the preliminary
212 purification and identification of CircRNA [46].
213   As a first step, the processing of extracted RNA with exonucleases such as RNase R,
214 nicotinic acid phosphatase and 5 'end exonuclease can destroy most linear RNA, but circRNA
215 remain intact due to no open ends in circRNAs for these enzyme reactions. The circRNA specific
216 divergent primer can be used to amplify abundant circRNA in which linear RNAs do not amplify
217 [46,94]. Second, the migration velocity of circRNA is slower than the long linear RNA due to lack
218 of polarity at the end. In particularly, their migration is much slower than RNA from
219 homologous gene transcription in the weak crosslinked gel. This difference helps to detect
220 them easily through Northern blot analysis [95]. Third, the fluorescence in situ hybridization
221 technique can locate circRNA in subcellular level [7,8]. As the circRNA do not have poly (A)
222 structure, the traditional oligo dT enrichment method, using ribo - zero kit to remove rRNA, is
223 not to be effective. The removal of linear RNA using RNase R is a most effective for the
224 enrichment of circRNA and library building [46,96].

225 6. 1. 2 High-throughput sequencing

226   The traditional RNA-seq technique does not distinguish the circRNA from linear RNAs. As a
227 result, the researchers have made some effective improvements in order to detect and validate
228 circRNA. First, as the intergenic exon rearrangement has different forms, building the divergent
229 primers with boundary combination can form circRNA candidate sequence and then it can be
230 compared with the sequencing data [97]. Second, the bioinformatic analysis of whole genome

231   sequence and assessement of sequence data through different sequence alignment algorithm
232   [14];.Third, the designing of templates with multiple sequence splice joint, which can directly
233   detect circRNA from the cDNA sequence [98]. Currently, many algorithms are available for the
234   prediction and studying circRNA that includes Acfs [99], FUCHS [100], CIRI2 [101], etc. Acfs
235   allows accurate and fast identification of circRNA. It also helps to determine the abundance of
236   circRNA from single- and paired-ended RNA-Seq data. It is well suitable for a wide spectrum of
237   applications including characterizing the landscape of circRNA from a variety of organisms.
238   FUCHS system is based on long sequencing reads (> 150bp / Reads), which achieves the
239   detection of a circular RNA within the variable shear and other information for more accurate
240   interpretation. CIRI2 uses the maximum likelihood estimate based on multiple seed matches to
241   identify the reverse splice junctions and it filters out false positives and mapping errors derived
242   from the repetitive sequence. CIRI2 has a significant balance of sensitivity, reliability, duration
243   and RAM usage[46, 96].

244   6. 1. 3 Gene chip

245   The human genome array, 133plus2.0 tool, can detect mRNA, but it does not able to detect
246   the ring RNA because the probe is designed for linear RNA. So it cannot effectively distinguish
247   cyclic RNA and linear RNA, when normal probe is used. However, if the probe is designed based
248   on the reverse splice site of the circRNA, this array tool specifically detect circRNA, as there is
249   no reverse splice site sequence on the linear RNA. In this way, it can effectively distinguish
250   circular RNA and mRNA.

251   6. 1. 4 Primer Design

252   The field of circRNA research gaining more attention recently due to the fact that they
253   contribute to many physiological as well as pathological processes. Unlike conventional PCR
254   primers, the designing of the circRNA primers should consider the following criteria: For the
255   detection of exonic circRNA, the primers for cross-cut site (backsplice) should be designed. In
256   the case of intron cyclized cyclRNAs, the primers targeting cross-cleavable sites should be used.
257   The primers can also be designed around intron regions. The length of the amplified product
258   should not be more than 100 bp. The squence position transformation is also important. The
259   difference in selection of primers for linear RNA and circRNA is given in Fig. 4.
260   The actual amplification effect after primer design needs to be experimentally determined.
261   If the quantification of circRNA is carried out by qPCR, the length of amplification should be
262   settled according to the experimental requirements of qPCR. Thus, qPCR remains the most
263   widely used technique to assess the expression level of circRNA.

264   *6. 2 The Database in CircRNA Research*

265   In recent years, the number of cirRNA research tools is rapidly increasing with different
266   aspects and improved functional analysis. Here, we present currently available online databases
267   for the detection and characterization of circRNA, which contain GenBank annotations or
268   circRNA from published articles. Each database analyses cricRNA with different characteristics
269   features feeded for the detection and they provide much information about circRNA. There are
270   several free online databases available for the circRNA research (Table 3).

8. Conclusion and Future Directions

CircRNAs have the characteristics of conserved sequence, tissue specificity, more stability and high abundance of expression, which enables them to be potential markers for disease screening and treatment. The rapid development of high throughput sequencing techniques and bioinformatic analyses suggest that it is likely to become a new efficient target in the clinical settings for the detection and treatment of diseases such as diabetes, cancer, cardiovascular disease and neurological diseases. It is notable that circRNAs could function as miRNA sponges and regulating multiple signaling pathways in the cardiovascular diseases, different types of cancer, neurodegenerative diseases and diabetes. However, further researches are needed to reveal the complete biological functions of of circRNA in terms of both physiological and pathological processes so that it can be applied to clinical use in future.

Despite the rapid advancement has been in the detection and characterization of circRNA, the knowledge about the functions of circular RNA is still at an early stage, which is one of the major drawbacks for the potential use of circRNA for therapeutic or diagnostic purposes. The new generation methods such as chip technology can be used to screen the possible disease-related circRNA in the cell or experimental animal models. This will increase our knowledge about the role of circRNA in the occurrence and development of pathological disorders. In addition to deepening the circRNA research in functional aspects, the someother questions should also to be refined. For example, how the dynamics of the circRNA formation is triggered and controlled? What is the link between circRNA formation process and the corresponding linear RNA generation? What is the relationship between different circRNA products from the same gene? How is the circRNA generation precisely regulated? The identification and characterisation of specific circRNA interacting molecules is important to answer most of these fundamendal questions. In addition, the naming of circRNA has not yet been unified and the mechanisms of circRNA in many diseases are not clear. By solving all these questions, circRNA could be a promising diagnostic tool for the detection and efficient therapeutic targets for treatment of various pathological disorders.

Conflicts of Interest: All the authors do not have any possible conficts of interest.

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References

[1]. Wilusz JE, Sharp PA (2013) Molecular biology. A circuitous route to noncoding RNA. Science 340 (6131):440-441. doi:10.1126/science.1238522

[2]. Yang Y, Fan X, Mao M, Song X, Wu P, Zhang Y, Jin Y, Yang Y, Chen LL, Wang Y, Wong CC, Xiao X, Wang Z (2017) Extensive translation of circular RNAs driven by N6-methyladenosine. Cell Res 27 (5):626-641. doi:10.1038/cr.2017.31

[3]. Yang Y, Gao X, Zhang M, Yan S, Sun C, Xiao F, Huang N, Yang X, Zhao K, Zhou H, Huang S, Xie B, Zhang N (2018) Novel Role of FBXW7 Circular RNA in Repressing Glioma Tumorigenesis. J Natl Cancer Inst 110 (3). doi:10.1093/jnci/djx166

[4]. Pamudurti NR, Bartok O, Jens M, Ashwal-Fluss R, Stottmeister C, Ruhe L, Hanan M, Wyler E, Perez-Hernandez D, Ramberger E, Shenzis S, Samson M, Dittmar G, Landthaler M, Chekulaeva M, Rajewsky N, Kadener S (2017) Translation of CircRNAs. Mol Cell 66 (1):9-21.e27. doi:10.1016/j.molcel.2017.02.021

[5]. Legnini I, Di Timoteo G, Rossi F, Morlando M, Briganti F, Sthandier O, Fatica A, Santini T, Andronache A, Wade M, Laneve P, Rajewsky N, Bozzoni I (2017) Circ-ZNF609 Is a Circular RNA that Can Be Translated and Functions in Myogenesis. Mol Cell 66 (1):22-37.e29. doi:10.1016/j.molcel.2017.02.017

[6]. Zhang XO, Wang HB, Zhang Y, Lu X, Chen LL, Yang L (2014) Complementary sequence-mediated exon circularization. Cell 159 (1):134-147. doi:10.1016/j.cell.2014.09.001

[7]. Zhang Y, Zhang XO, Chen T, Xiang JF, Yin QF, Xing YH, Zhu S, Yang L, Chen LL (2013) Circular intronic long noncoding RNAs. Mol Cell 51 (6):792-806. doi:10.1016/j.molcel.2013.08.017

[8]. Li Z, Huang C, Bao C, Chen L, Lin M, Wang X, Zhong G, Yu B, Hu W, Dai L, Zhu P, Chang Z, Wu Q, Zhao Y, Jia Y, Xu P, Liu H, Shan G (2017) Corrigendum: Exon-intron circular RNAs regulate transcription in the nucleus. Nat Struct Mol Biol 24 (2):194. doi:10.1038/nsmb0217-194a

[9]. Danan M, Schwartz S, Edelheit S, Sorek R (2012) Transcriptome-wide discovery of circular RNAs in Archaea. Nucleic Acids Res 40 (7):3131-3142. doi:10.1093/nar/gkr1009

[10]. Wang PL, Bao Y, Yee MC, Barrett SP, Hogan GJ, Olsen MN, Dinneny JR, Brown PO, Salzman J (2014) Circular RNA is expressed across the eukaryotic tree of life. PLoS One 9 (6):e90859. doi:10.1371/journal.pone.0090859

[11]. Morris KV, Mattick JS (2014) The rise of regulatory RNA. Nat Rev Genet 15 (6):423-437. doi:10.1038/nrg3722

[12]. Burd CE, Jeck WR, Liu Y, Sanoff HK, Wang Z, Sharpless NE (2010) Expression of linear and novel circular forms of an INK4/ARF-associated non-coding RNA correlates with atherosclerosis risk. PLoS Genet 6 (12):e1001233. doi:10.1371/journal.pgen.1001233

[13]. Wang Y, Mo Y, Gong Z, Yang X, Yang M, Zhang S, Xiong F, Xiang B, Zhou M, Liao Q, Zhang W, Li X, Li X, Li Y, Li G, Zeng Z, Xiong W (2017) Circular RNAs in human cancer. Mol Cancer 16 (1):25. doi:10.1186/s12943-017-0598-7

[14]. Jeck WR, Sorrentino JA, Wang K, Slevin MK, Burd CE, Liu J, Marzluff WF, Sharpless NE (2013) Circular RNAs are abundant, conserved, and associated with ALU repeats. RNA 19 (2):141-157. doi:10.1261/rna.035667.112

[15]. Huang C, Shan G (2015) What happens at or after transcription: Insights into circRNA biogenesis and function. Transcription 6 (4):61-64. doi:10.1080/21541264.2015.1071301

[16]. Li F, Zhang L, Li W, Deng J, Zheng J, An M, Lu J, Zhou Y (2015) Circular RNA ITCH has inhibitory effect on ESCC by suppressing the Wnt/beta-catenin pathway. Oncotarget 6 (8):6001-6013. doi:10.18632/oncotarget.3469

348   [17]. Rodriguez-Trelles F, Tarrio R, Ayala FJ (2006) Origins and evolution of spliceosomal introns. Annu Rev Genet
349   40:47-76. doi:10.1146/annurev.genet.40.110405.090625
350   [18]. Hansen TB, Jensen TI, Clausen BH, Bramsen JB, Finsen B, Damgaard CK, Kjems J (2013) Natural RNA circles
351   function as efficient microRNA sponges. Nature 495 (7441):384-388. doi:10.1038/nature11993
352   [19]. Aktas T, Avsar Ilik I, Maticzka D, Bhardwaj V, Pessoa Rodrigues C, Mittler G, Manke T, Backofen R, Akhtar A
353   (2017) DHX9 suppresses RNA processing defects originating from the Alu invasion of the human genome. Nature
354   544 (7648):115-119. doi:10.1038/nature21715
355   [20]. Shen T, Han M, Wei G, Ni T (2015) An intriguing RNA species--perspectives of circularized RNA. Protein Cell 6
356   (12):871-880. doi:10.1007/s13238-015-0202-0
357   [21]. Li X, Liu CX, Xue W, Zhang Y, Jiang S, Yin QF, Wei J, Yao RW, Yang L, Chen LL (2017) Coordinated circRNA
358   Biogenesis and Function with NF90/NF110 in Viral Infection. Mol Cell. doi:10.1016/j.molcel.2017.05.023
359   [22]. Fei T, Chen Y, Xiao T, Li W, Cato L, Zhang P, Cotter MB, Bowden M, Lis RT, Zhao SG, Wu Q, Feng FY, Loda M, He
360   HH, Liu XS, Brown M (2017) Genome-wide CRISPR screen identifies HNRNPL as a prostate cancer dependency
361   regulating RNA splicing. Proc Natl Acad Sci U S A 114 (26):E5207-e5215. doi:10.1073/pnas.1617467114
362   [23]. Ashwal-Fluss R, Meyer M, Pamudurti NR, Ivanov A, Bartok O, Hanan M, Evantal N, Memczak S, Rajewsky N,
363   Kadener S (2014) circRNA biogenesis competes with pre-mRNA splicing. Mol Cell 56 (1):55-66.
364   doi:10.1016/j.molcel.2014.08.019
365   [24]. Conn SJ, Pillman KA, Toubia J, Conn VM, Salmanidis M, Phillips CA, Roslan S, Schreiber AW, Gregory PA,
366   Goodall GJ (2015) The RNA binding protein quaking regulates formation of circRNAs. Cell 160 (6):1125-1134.
367   doi:10.1016/j.cell.2015.02.014
368   [25]. Errichelli L, Dini Modigliani S, Laneve P, Colantoni A, Legnini I, Capauto D, Rosa A, De Santis R, Scarfo R,
369   Peruzzi G, Lu L, Caffarelli E, Shneider NA, Morlando M, Bozzoni I (2017) FUS affects circular RNA expression in
370   murine embryonic stem cell-derived motor neurons. Nature communications 8:14741. doi:10.1038/ncomms14741
371   [26]. Ivanov A, Memczak S, Wyler E, Torti F, Porath HT, Orejuela MR, Piechotta M, Levanon EY, Landthaler M,
372   Dieterich C, Rajewsky N (2015) Analysis of intron sequences reveals hallmarks of circular RNA biogenesis in animals.
373   Cell Rep 10 (2):170-177. doi:10.1016/j.celrep.2014.12.019
374   [27]. Liang D, Tatomer DC, Luo Z, Wu H, Yang L, Chen LL, Cherry S, Wilusz JE (2017) The Output of Protein-Coding
375   Genes Shifts to Circular RNAs When the Pre-mRNA Processing Machinery Is Limiting. Mol Cell 68 (5):940-954.e943.
376   doi:10.1016/j.molcel.2017.10.034
377   [28]. Liu J, Liu T, Wang X, He A (2017) Circles reshaping the RNA world: from waste to treasure. Mol Cancer 16
378   (1):58. doi:10.1186/s12943-017-0630-y
379   [29]. Cocquerelle C, Mascrez B, Hetuin D, Bailleul B (1993) Mis-splicing yields circular RNA molecules. FASEB J 7
380   (1):155-160
381   [30]. Yang C, Wu D, Gao L, Liu X, Jin Y, Wang D, Wang T, Li X (2016) Competing endogenous RNA networks in
382   human cancer: hypothesis, validation, and perspectives. Oncotarget 7 (12):13479-13490.
383   doi:10.18632/oncotarget.7266
384   [31]. Salzman J, Gawad C, Wang PL, Lacayo N, Brown PO (2012) Circular RNAs are the predominant transcript
385   isoform from hundreds of human genes in diverse cell types. PLoS One 7 (2):e30733.
386   doi:10.1371/journal.pone.0030733

387   [32]. Memczak S, Jens M, Elefsinioti A, Torti F, Krueger J, Rybak A, Maier L, Mackowiak SD, Gregersen LH,

388   Munschauer M, Loewer A, Ziebold U, Landthaler M, Kocks C, le Noble F, Rajewsky N (2013) Circular RNAs are a

389   large class of animal RNAs with regulatory potency. Nature 495 (7441):333-338. doi:10.1038/nature11928

390   [33]. Qu S, Yang X, Li X, Wang J, Gao Y, Shang R, Sun W, Dou K, Li H (2015) Circular RNA: A new star of noncoding

391   RNAs. Cancer Lett 365 (2):141-148. doi:10.1016/j.canlet.2015.06.003

392   [34]. Bahn JH, Zhang Q, Li F, Chan TM, Lin X, Kim Y, Wong DT, Xiao X (2015) The landscape of microRNA, Piwi-

393   interacting RNA, and circular RNA in human saliva. Clin Chem 61 (1):221-230. doi:10.1373/clinchem.2014.230433

394   [35]. Rybak-Wolf A, Stottmeister C, Glazar P, Jens M, Pino N, Giusti S, Hanan M, Behm M, Bartok O, Ashwal-Fluss R,

395   Herzog M, Schreyer L, Papavasileiou P, Ivanov A, Ohman M, Refojo D, Kadener S, Rajewsky N (2015) Circular RNAs

396   in the Mammalian Brain Are Highly Abundant, Conserved, and Dynamically Expressed. Mol Cell 58 (5):870-885.

397   doi:10.1016/j.molcel.2015.03.027

398   [36]. Suzuki H, Tsukahara T (2014) A view of pre-mRNA splicing from RNase R resistant RNAs. International journal

399   of molecular sciences 15 (6):9331-9342. doi:10.3390/ijms15069331

400   [37]. Zhao ZJ, Shen J (2015) Circular RNA participates in the carcinogenesis and the malignant behavior of cancer.

401   RNA Biol:1-8. doi:10.1080/15476286.2015.1122162

402   [38]. Hansen TB, Kjems J, Damgaard CK (2013) Circular RNA and miR-7 in cancer. Cancer Res 73 (18):5609-5612.

403   doi:10.1158/0008-5472.can-13-1568

404   [39]. Barrett SP, Parker KR, Horn C, Mata M, Salzman J (2017) ciRS-7 exonic sequence is embedded in a long non-

405   coding RNA locus. PLoS Genet 13 (12):e1007114. doi:10.1371/journal.pgen.1007114

406   [40]. Zheng Q, Bao C, Guo W, Li S, Chen J, Chen B, Luo Y, Lyu D, Li Y, Shi G, Liang L, Gu J, He X, Huang S (2016)

407   Circular RNA profiling reveals an abundant circHIPK3 that regulates cell growth by sponging multiple miRNAs.

408   Nature communications 7:11215. doi:10.1038/ncomms11215

409   [41]. Huang R, Zhang Y, Han B, Bai Y, Zhou R, Gan G, Chao J, Hu G, Yao H (2017) Circular RNA HIPK2 regulates

410   astrocyte activation via cooperation of autophagy and ER stress by targeting MIR124-2HG. Autophagy 13

411   (10):1722-1741. doi:10.1080/15548627.2017.1356975

412   [42]. Yang L, Han B, Zhang Y, Bai Y, Chao J, Hu G, Yao H (2018) Engagement of circular RNA HECW2 in the

413   nonautophagic role of ATG5 implicated in the endothelial-mesenchymal transition. Autophagy:1-15.

414   doi:10.1080/15548627.2017.1414755

415   [43]. Chen LL (2016) The biogenesis and emerging roles of circular RNAs. Nat Rev Mol Cell Biol 17 (4):205-211.

416   doi:10.1038/nrm.2015.32

417   [44]. Tay ML, Pek JW (2017) Maternally Inherited Stable Intronic Sequence RNA Triggers a Self-Reinforcing

418   Feedback Loop during Development. Curr Biol 27 (7):1062-1067. doi:10.1016/j.cub.2017.02.040

419   [45]. Hentze MW, Preiss T (2013) Circular RNAs: splicing's enigma variations. EMBO J 32 (7):923-925.

420   doi:10.1038/emboj.2013.53

421   [46]. Jeck WR, Sharpless NE (2014) Detecting and characterizing circular RNAs. Nat Biotechnol 32 (5):453-461.

422   doi:10.1038/nbt.2890

423   [47]. Chen YG, Kim MV, Chen X, Batista PJ, Aoyama S, Wilusz JE, Iwasaki A, Chang HY (2017) Sensing Self and

424   Foreign Circular RNAs by Intron Identity. Mol Cell. doi:10.1016/j.molcel.2017.05.022

425   [48]. Abe N, Matsumoto K, Nishihara M, Nakano Y, Shibata A, Maruyama H, Shuto S, Matsuda A, Yoshida M, Ito Y,

426   Abe H (2015) Rolling Circle Translation of Circular RNA in Living Human Cells. Scientific reports 5:16435.

427   doi:10.1038/srep16435

428   [49]. Wang Y, Wang Z (2015) Efficient backsplicing produces translatable circular mRNAs. RNA 21 (2):172-179.

429   doi:10.1261/rna.048272.114

430   [50]. Thompson SR (2012) So you want to know if your message has an IRES? Wiley Interdiscip Rev RNA 3 (5):697-

431   705. doi:10.1002/wrna.1129

432   [51]. Dong R, Zhang XO, Zhang Y, Ma XK, Chen LL, Yang L (2016) CircRNA-derived pseudogenes. Cell Res 26 (6):747-

433   750. doi:10.1038/cr.2016.42

434   [52]. Kalyana-Sundaram S, Kumar-Sinha C, Shankar S, Robinson DR, Wu YM, Cao X, Asangani IA, Kothari V, Prensner

435   JR, Lonigro RJ, Iyer MK, Barrette T, Shanmugam A, Dhanasekaran SM, Palanisamy N, Chinnaiyan AM (2012)

436   Expressed pseudogenes in the transcriptional landscape of human cancers. Cell 149 (7):1622-1634.

437   doi:10.1016/j.cell.2012.04.041

438   [53]. Wang YS, Zhou J, Hong K, Cheng XS, Li YG (2015) MicroRNA-223 displays a protective role against

439   cardiomyocyte hypertrophy by targeting cardiac troponin I-interacting kinase. Cell Physiol Biochem 35 (4):1546-

440   1556. doi:10.1159/000373970

441   [54]. Wang K, Long B, Liu F, Wang JX, Liu CY, Zhao B, Zhou LY, Sun T, Wang M, Yu T, Gong Y, Liu J, Dong YH, Li N, Li

442   PF (2016) A circular RNA protects the heart from pathological hypertrophy and heart failure by targeting miR-223.

443   Eur Heart J 37 (33):2602-2611. doi:10.1093/eurheartj/ehv713

444   [55]. Holdt LM, Stahringer A, Sass K, Pichler G, Kulak NA, Wilfert W, Kohlmaier A, Herbst A, Northoff BH, Nicolaou A,

445   Gabel G, Beutner F, Scholz M, Thiery J, Musunuru K, Krohn K, Mann M, Teupser D (2016) Circular non-coding RNA

446   ANRIL modulates ribosomal RNA maturation and atherosclerosis in humans. Nature communications 7:12429.

447   doi:10.1038/ncomms12429

448   [56]. Du WW, Yang W, Chen Y, Wu ZK, Foster FS, Yang Z, Li X, Yang BB (2016) Foxo3 circular RNA promotes cardiac

449   senescence by modulating multiple factors associated with stress and senescence responses. Eur Heart J.

450   doi:10.1093/eurheartj/ehw001

451   [57]. Du WW, Yang W, Liu E, Yang Z, Dhaliwal P, Yang BB (2016) Foxo3 circular RNA retards cell cycle progression

452   via forming ternary complexes with p21 and CDK2. Nucleic Acids Res 44 (6):2846-2858. doi:10.1093/nar/gkw027

453   [58]. Geng HH, Li R, Su YM, Xiao J, Pan M, Cai XX, Ji XP (2016) The Circular RNA Cdr1as Promotes Myocardial

454   Infarction by Mediating the Regulation of miR-7a on Its Target Genes Expression. PLoS One 11 (3):e0151753.

455   doi:10.1371/journal.pone.0151753

456   [59]. Li B, Li R, Zhang C, Bian HJ, Wang F, Xiao J, Liu SW, Yi W, Zhang MX, Wang SX, Zhang Y, Su GH, Ji XP (2014)

457   MicroRNA-7a/b protects against cardiac myocyte injury in ischemia/reperfusion by targeting poly(ADP-ribose)

458   polymerase. PLoS One 9 (3):e90096. doi:10.1371/journal.pone.0090096

459   [60]. Read DE, Gupta A, Ladilov Y, Samali A, Gupta S (2014) miRNA signature of unfolded protein response in H9c2

460   rat cardiomyoblasts. Cell Biosci 4 (1):56. doi:10.1186/2045-3701-4-56

461   [61]. Wang K, Gan TY, Li N, Liu CY, Zhou LY, Gao JN, Chen C, Yan KW, Ponnusamy M, Zhang YH, Li PF (2017) Circular

462   RNA mediates cardiomyocyte death via miRNA-dependent upregulation of MTP18 expression. Cell Death Differ 24

463   (6):1111-1120. doi:10.1038/cdd.2017.61

464   [62]. Nan A, Chen L, Zhang N, Liu Z, Yang T, Wang Z, Yang C, Jiang Y (2017) A novel regulatory network among

465   LncRpa, CircRar1, MiR-671 and apoptotic genes promotes lead-induced neuronal cell apoptosis. Arch Toxicol 91

466   (4):1671-1684. doi:10.1007/s00204-016-1837-1

467   [63]. Wu Y, Zhang Y, Zhang Y, Wang JJ (2017) CircRNA hsa_circ_0005105 up regulates NAMPT expression and

468   promotes chondrocyte extracellular matrix degradation by sponging miR-26a. Cell Biol Int. doi:10.1002/cbin.10761

469   [64]. Cui X, Niu W, Kong L, He M, Jiang K, Chen S, Zhong A, Li W, Lu J, Zhang L (2016) hsa_circRNA_103636:

470   potential novel diagnostic and therapeutic biomarker in Major depressive disorder. Biomark Med 10 (9):943-952.

471   doi:10.2217/bmm-2016-0130

472   [65]. Zhou Z, Jiang R, Yang X, Guo H, Fang S, Zhang Y, Cheng Y, Wang J, Yao H, Chao J (2018) circRNA Mediates

473   Silica-Induced Macrophage Activation Via HECTD1/ZC3H12A-Dependent Ubiquitination. Theranostics 8 (2):575-592.

474   doi:10.7150/thno.21648

475   [66]. Kim MK, Shin HM, Jung H, Lee E, Kim TK, Kim TN, Kwon MJ, Lee SH, Rhee BD, Park JH (2017) Comparison of

476   pancreatic beta cells and alpha cells under hyperglycemia: Inverse coupling in pAkt-FoxO1. Diabetes Res Clin Pract

477   131:1-11. doi:10.1016/j.diabres.2017.05.017

478   [67]. Xu H, Guo S, Li W, Yu P (2015) The circular RNA Cdr1as, via miR-7 and its targets, regulates insulin

479   transcription and secretion in islet cells. Scientific reports 5:12453. doi:10.1038/srep12453

480   [68]. Wang Y, Liu J, Liu C, Naji A, Stoffers DA (2013) MicroRNA-7 regulates the mTOR pathway and proliferation in

481   adult pancreatic beta-cells. Diabetes 62 (3):887-895. doi:10.2337/db12-0451

482   [69]. Li P, Chen S, Chen H, Mo X, Li T, Shao Y, Xiao B, Guo J (2015) Using circular RNA as a novel type of biomarker

483   in the screening of gastric cancer. Clin Chim Acta 444:132-136. doi:10.1016/j.cca.2015.02.018

484   [70]. Zhang Y, Liu H, Li W, Yu J, Li J, Shen Z, Ye G, Qi X, Li G (2017) CircRNA_100269 is downregulated in gastric

485   cancer and suppresses tumor cell growth by targeting miR-630. Aging (Albany NY). doi:10.18632/aging.101254

486   [71]. Tian M, Chen R, Li T, Xiao B (2017) Reduced expression of circRNA hsa_circ_0003159 in gastric cancer and its

487   clinical significance. J Clin Lab Anal. doi:10.1002/jcla.22281

488   [72]. Shao Y, Chen L, Lu R, Zhang X, Xiao B, Ye G, Guo J (2017) Decreased expression of hsa_circ_0001895 in human

489   gastric   cancer   and   its   clinical   significances.   Tumour   Biol   39   (4):1010428317699125.

490   doi:10.1177/1010428317699125

491   [73]. Chen J, Li Y, Zheng Q, Bao C, He J, Chen B, Lyu D, Zheng B, Xu Y, Long Z, Zhou Y, Zhu H, Wang Y, He X, Shi Y,

492   Huang S (2017) Circular RNA profile identifies circPVT1 as a proliferative factor and prognostic marker in gastric

493   cancer. Cancer Lett 388:208-219. doi:10.1016/j.canlet.2016.12.006

494   [74]. Wang X, Zhang Y, Huang L, Zhang J, Pan F, Li B, Yan Y, Jia B, Liu H, Li S, Zheng W (2015) Decreased expression

495   of hsa_circ_001988 in colorectal cancer and its clinical significances. Int J Clin Exp Pathol 8 (12):16020-16025

496   [75]. Xie H, Ren X, Xin S, Lan X, Lu G, Lin Y, Yang S, Zeng Z, Liao W, Ding YQ, Liang L (2016) Emerging roles of

497   circRNA_001569 targeting miR-145 in the proliferation and invasion of colorectal cancer. Oncotarget 7 (18):26680-

498   26691. doi:10.18632/oncotarget.8589

499   [76]. Guo JN, Li J, Zhu CL, Feng WT, Shao JX, Wan L, Huang MD, He JD (2016) Comprehensive profile of differentially

500   expressed circular RNAs reveals that hsa_circ_0000069 is upregulated and promotes cell proliferation, migration,

501   and invasion in colorectal cancer. Onco Targets Ther 9:7451-7458. doi:10.2147/ott.s123220

502   [77]. Hsiao KY, Lin YC, Gupta SK, Chang N, Yen L, Sun HS, Tsai SJ (2017) Noncoding Effects of Circular RNA CCDC66

503   Promote Colon Cancer Growth and Metastasis. Cancer Res 77 (9):2339-2350. doi:10.1158/0008-5472.can-16-1883

504   [78]. Xia W, Qiu M, Chen R, Wang S, Leng X, Wang J, Xu Y, Hu J, Dong G, Xu PL, Yin R (2016) Circular RNA
505   has_circ_0067934 is upregulated in esophageal squamous cell carcinoma and promoted proliferation. Scientific
506   reports 6:35576. doi:10.1038/srep35576

507   [79]. Xu L, Zhang M, Zheng X, Yi P, Lan C, Xu M (2017) The circular RNA ciRS-7 (Cdr1as) acts as a risk factor of
508   hepatic microvascular invasion in hepatocellular carcinoma. J Cancer Res Clin Oncol 143 (1):17-27.
509   doi:10.1007/s00432-016-2256-7

510   [80]. Shang X, Li G, Liu H, Li T, Liu J, Zhao Q, Wang C (2016) Comprehensive Circular RNA Profiling Reveals That
511   hsa_circ_0005075, a New Circular RNA Biomarker, Is Involved in Hepatocellular Crcinoma Development. Medicine
512   (Baltimore) 95 (22):e3811. doi:10.1097/md.0000000000003811

513   [81]. Fu L, Yao T, Chen Q, Mo X, Hu Y, Guo J (2017) Screening differential circular RNA expression profiles reveals
514   hsa_circ_0004018 is associated with hepatocellular carcinoma. Oncotarget. doi:10.18632/oncotarget.16881

515   [82]. Qin M, Liu G, Huo X, Tao X, Sun X, Ge Z, Yang J, Fan J, Liu L, Qin W (2016) Hsa_circ_0001649: A circular RNA
516   and potential novel biomarker for hepatocellular carcinoma. Cancer Biomark 16 (1):161-169. doi:10.3233/cbm-
517   150552

518   [83]. Guo W, Zhang J, Zhang D, Cao S, Li G, Zhang S, Wang Z, Wen P, Yang H, Shi X, Pan J, Ye H (2017)
519   Polymorphisms and expression pattern of circular RNA circ-ITCH contributes to the carcinogenesis of
520   hepatocellular carcinoma. Oncotarget. doi:10.18632/oncotarget.18327

521   [84]. Shi L, Yan P, Liang Y, Sun Y, Shen J, Zhou S, Lin H, Liang X, Cai X (2017) Circular RNA expression is suppressed
522   by androgen receptor (AR)-regulated adenosine deaminase that acts on RNA (ADAR1) in human hepatocellular
523   carcinoma. Cell death & disease 8 (11):e3171. doi:10.1038/cddis.2017.556

524   [85]. Lee BY, Timpson P, Horvath LG, Daly RJ (2015) FAK signaling in human cancer as a target for therapeutics.
525   Pharmacol Ther 146:132-149. doi:10.1016/j.pharmthera.2014.10.001

526   [86]. Ma HB, Yao YN, Yu JJ, Chen XX, Li HF (2018) Extensive profiling of circular RNAs and the potential regulatory
527   role of circRNA-000284 in cell proliferation and invasion of cervical cancer via sponging miR-506. American journal
528   of translational research 10 (2):592-604

529   [87]. Lu L, Sun J, Shi P, Kong W, Xu K, He B, Zhang S, Wang J (2017) Identification of circular RNAs as a promising
530   new class of diagnostic biomarkers for human breast cancer. Oncotarget. doi:10.18632/oncotarget.17307

531   [88]. Couderc C, Boin A, Fuhrmann L, Vincent-Salomon A, Mandati V, Kieffer Y, Mechta-Grigoriou F, Del Maestro L,
532   Chavrier P, Vallerand D, Brito I, Dubois T, De Koning L, Bouvard D, Louvard D, Gautreau A, Lallemand D (2016)
533   AMOTL1 Promotes Breast Cancer Progression and Is Antagonized by Merlin. Neoplasia 18 (1):10-24.
534   doi:10.1016/j.neo.2015.11.010

535   [89]. Yang Q, Du WW, Wu N, Yang W, Awan FM, Fang L, Ma J, Li X, Zeng Y, Yang Z, Dong J, Khorshidi A, Yang BB
536   (2017) A circular RNA promotes tumorigenesis by inducing c-myc nuclear translocation. Cell Death Differ.
537   doi:10.1038/cdd.2017.86

538   [90]. Chen L, Zhang S, Wu J, Cui J, Zhong L, Zeng L, Ge S (2017) circRNA_100290 plays a role in oral cancer by
539   functioning as a sponge of the miR-29 family. Oncogene. doi:10.1038/onc.2017.89

540   [91]. Zhu X, Wang X, Wei S, Chen Y, Chen Y, Fan X, Han S, Wu G (2017) hsa_circ_0013958: a circular RNA and
541   potential novel biomarker for lung adenocarcinoma. FEBS J. doi:10.1111/febs.14132

542   [92]. Zhong Z, Lv M, Chen J (2016) Screening differential circular RNA expression profiles reveals the regulatory role

543   of circTCF25-miR-103a-3p/miR-107-CDK6 pathway in bladder carcinoma. Scientific reports 6:30919.

544   doi:10.1038/srep30919

545   [93]. Xu ZQ, Yang MG, Liu HJ, Su CQ (2017) Circular RNA hsa_circ_0003221 (circPTK2) promotes the proliferation

546   and migration of bladder cancer cells. J Cell Biochem. doi:10.1002/jcb.26492

547   [94]. Suzuki H, Zuo Y, Wang J, Zhang MQ, Malhotra A, Mayeda A (2006) Characterization of RNase R-digested

548   cellular RNA source that consists of lariat and circular RNAs from pre-mRNA splicing. Nucleic Acids Res 34 (8):e63.

549   doi:10.1093/nar/gkl151

550   [95]. Tabak HF, Van der Horst G, Smit J, Winter AJ, Mul Y, Groot Koerkamp MJ (1988) Discrimination between RNA

551   circles, interlocked RNA circles and lariats using two-dimensional polyacrylamide gel electrophoresis. Nucleic Acids

552   Res 16 (14a):6597-6605

553   [96]. Ebbesen KK, Kjems J, Hansen TB (2016) Circular RNAs: Identification, biogenesis and function. Biochim

554   Biophys Acta 1859 (1):163-168. doi:10.1016/j.bbagrm.2015.07.007

555   [97]. Salzman J, Chen RE, Olsen MN, Wang PL, Brown PO (2013) Cell-type specific features of circular RNA

556   expression. PLoS Genet 9 (9):e1003777. doi:10.1371/journal.pgen.1003777

557   [98]. Hoffmann S, Otto C, Doose G, Tanzer A, Langenberger D, Christ S, Kunz M, Holdt LM, Teupser D, Hackermuller

558   J, Stadler PF (2014) A multi-split mapping algorithm for circular RNA, splicing, trans-splicing and fusion detection.

559   Genome biology 15 (2):R34. doi:10.1186/gb-2014-15-2-r34

560   [99]. You X, Conrad TO (2016) Acfs: accurate circRNA identification and quantification from RNA-Seq data.

561   Scientific reports 6:38820. doi:10.1038/srep38820

562   [100]. Metge F, Czaja-Hasse LF, Reinhardt R, Dieterich C (2017) FUCHS-towards full circular RNA characterization

563   using RNAseq. PeerJ 5:e2934. doi:10.7717/peerj.2934

564   [101]. Gao Y, Zhang J, Zhao F (2017) Circular RNA identification based on multiple seed matching. Brief Bioinform.

565   doi:10.1093/bib/bbx014

566   [102]. Wu SM, Liu H, Huang PJ, Chang IY, Lee CC, Yang CY, Tsai WS, Tan BC (2018) circlncRNAnet: an integrated

567   web-based resource for mapping functional networks of long or circular forms of noncoding RNAs. GigaScience 7

568   (1):1-10. doi:10.1093/gigascience/gix118

569   [103]. Li JH, Liu S, Zhou H, Qu LH, Yang JH (2014) starBase v2.0: decoding miRNA-ceRNA, miRNA-ncRNA and

570   protein-RNA interaction networks from large-scale CLIP-Seq data. Nucleic Acids Res 42 (Database issue):D92-97.

571   doi:10.1093/nar/gkt1248

572   [104]. Glazar P, Papavasileiou P, Rajewsky N (2014) circBase: a database for circular RNAs. RNA 20 (11):1666-1670.

573   doi:10.1261/rna.043687.113

574   [105]. Hancock JM (2014) Circles within circles: commentary on Ghosal et al. (2013) "Circ2Traits: a comprehensive

575   database for circular RNA potentially associated with disease and traits". Front Genet 5:459.

576   doi:10.3389/fgene.2014.00459

577   [106]. Ferdin J, Kunej T, Calin GA (2010) Non-coding RNAs: identification of cancer-associated microRNAs by gene

578   profiling. Technol Cancer Res Treat 9 (2):123-138. doi:10.1177/153303461000900202

579   [107]. Liu YC, Li JR, Sun CH, Andrews E, Chao RF, Lin FM, Weng SL, Hsu SD, Huang CC, Cheng C, Liu CC, Huang HD

580   (2016) CircNet: a database of circular RNAs derived from transcriptome sequencing data. Nucleic Acids Res 44

581   (D1):D209-215. doi:10.1093/nar/gkv940

582   [108]. Zheng LL, Li JH, Wu J, Sun WJ, Liu S, Wang ZL, Zhou H, Yang JH, Qu LH (2016) deepBase v2.0: identification,

583   expression, evolution and function of small RNAs, LncRNAs and circular RNAs from deep-sequencing data. Nucleic

584   Acids Res 44 (D1):D196-202. doi:10.1093/nar/gkv1273

585   [109]. Dudekula DB, Panda AC, Grammatikakis I, De S, Abdelmohsen K, Gorospe M (2016) CircInteractome: A web

586   tool for exploring circular RNAs and their interacting proteins and microRNAs. RNA Biol 13 (1):34-42.

587   doi:10.1080/15476286.2015.1128065

588   [110]. Xia S, Feng J, Lei L, Hu J, Xia L, Wang J, Xiang Y, Liu L, Zhong S, Han L, He C (2016) Comprehensive

589   characterization of tissue-specific circular RNAs in the human and mouse genomes. Brief Bioinform.

590   doi:10.1093/bib/bbw081

591   [111]. Zhang XO, Dong R, Zhang Y, Zhang JL, Luo Z, Zhang J, Chen LL, Yang L (2016) Diverse alternative back-splicing

592   and alternative splicing landscape of circular RNAs. Genome Res 26 (9):1277-1287. doi:10.1101/gr.202895.115

593   [112]. Chen X, Han P, Zhou T, Guo X, Song X, Li Y (2016) circRNADb: A comprehensive database for human circular

594   RNAs with protein-coding annotations. Scientific reports 6:34985. doi:10.1038/srep34985

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Table 1 (on next page)

The characteristics of different types of CircRNA

ecRNA : Exon circRNA ; ciRNA : Intron circRNA ; ElciRNA : Exon-intron circRNA

1   **Table 1** The characteristics of different types of CircRNA

Name	Type	Location	Joint Site	Sequence Feature	Function
ecRNA[6]	exon	cytoplasm	3'-5' phosphodiester bond	Formed by cyclization of exons containing the reverse complementary sequence of introns and selective cyclization.	Functioning as MiRNA Sponges; Interact with RNA Binding Proteins (RBPs); Participates in translation.
ciRNA[7]	intron	nucleus	2'-5' phosphodiester bond	5 'splice site enriched with 7 GU motif and 3' branch site contains 11 C motif.	Regulation of gene transcription.
ElciRNA[8]	exon - intron	nucleus	3'-5' phosphodiester bond	Formed by cyclization of exons containing the reverse complementary sequence of introns and selective cyclization.	Regulation of gene transcription.

2   ecRNA: Exon circRNA; ciRNA: Intron circRNA; ElciRNA: Exon-intron circRNA

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Table 2(on next page)

CircRNA in disease development and progression

HRCR: heart-related circRNA ; Canril: circular antisense non-coding RNA at the INK4 locus ; MFACR: mitochondrial fission and apoptosis-related circRNA;

1   Table 2 CircRNA in disease development and progression

Diseases		CircRNA	Functions
Cardiovascular Diseases	Pathological hypertrophy and heart failure (HF)	HRCR	Mir-223 is a positive modulator of hypertrophy in cardiomyocytes, which can induce cardiac hypertrophy and heart failure [49,53], HRCR acts as an endogenous mir-223 sponge to inhibit the hypertrophic response [54].
	Atherosclerosis	CANRIL	CANRIL prevents rRNA pre-binding and exonuclease-mediated rRNA maturation by binding to the C-terminal lysine-rich domain of PES1, this pathway can inhibit atherosclerosis by eliminating hyperproliferative cell types in atherosclerotic plaques [55].
	Cardiac senescence	Circ-Foxo3	Circ-Foxo3 is generated from Foxo3. Its expression is highly correlate with markers of cellular senescence [56] and circ-Foxo3 represses cell cycle progression and cell proliferation [57], it interacts with several transcription factors (E2F1, FAK, and HIF1a) and anti-senescent protein such as ID-1 and preventing their nucleus entry, which repress their anti-senescence roles, circ-Foxp3 also positively correlates with cellular senescence [56].
	Myocardial Infarction(MI)	Cdr1as	Cdr1as promotes apoptosis and MI injury by blocking the activity of mir-7a and increasing the expression of mir-7a targets such as PARP and SP1[58-60].
		MFACR	MFACR dependent inhibition of mir-652-3p increases MTP18 and mitochondrial fission, which results in reduction of cardiomyocyte apoptosis

			and extension of MI injury [61].
Neurodegenerative Diseases		CDR1as	In lead-induced neuronal apoptosis, circRar1 can directly inhibit miR-671, which leads to the suppression of Akt2 and increased expression of Caspase-8 and other apoptosis-related proteins [62].
Osteoarthritis (OA)		Hsa_circ_0005105	It promotes extracellular matrix (ECM) degradation by regulating the expression of mir-26a target NAMPT [63].
Major depressive disorders (MDD).		Hsa_circRNA_103636	It is easily detectable in blood samples and its expression pattern altered in MDD [64].
Silicosis		CircHECTD1	It mediates silica-induced macrophage activation via HECTD1/ZC3H12A-dependent ubiquitination [65].
Diabetes		Cdrlas	The impairment of Islet β-cell function leads to a absolute or relative insulin deficiency (insulin resistance), which causes increase in blood sugar level and diabetes [66,67], mir-7 negatively regulates the proliferation of islet β cells and targets multiple components of mammalian target of ripamycin (mtor) signaling pathway, which are involved in pancreatic β cell proliferation, these finding reveal that CDR1as/mir-7 could be a potential therapeutic target for treating and managing diabetes [68].
Cancer	Gastric Cancer (GC)	Hsa-circ-002059	The increased expression of Hsa-circ-002059 is significantly associated with the tumor stage of GC [69].
		Circrna_100269	Circrna_100269 can suppress gastric tumor cell growth by targeting mir-630. However, its expression is

			downregulated during GC [70].
		Hsa_circ_ 0003159	The expression of hsa_circ_0003159 is negatively associated with tumor-node-metastasis stage [71].
		Hsa_circ_ 0000190	The increased expression of hsa_circ_0001895 is significantly correlated with tissue carcino-embryonic antigen expression [72].
		CircPVT1	It promotes cell proliferation by acting as a sponge for members of miR-125 family and often upregulated in GC tissues [73].
	Colorectal Cancer (CRC)	Hsa_circ_ 001988	The expression of hsa_circ_001988 is downregulated and it is associated with differentiation and perineural invasion in CRC [74].
		Circ_0015 69	Circ_001569 directly inhibits the regulatory activity of mir-145, and thereby it up-regulates the expression of its protein targets such as E2F5, BAG4 and FMNL2 , which are involved in tumor proliferation and invasion in CRC [75].
		Hsa_circ_ 0000069	It upregulates cell proliferation, migration, and invasion [76].
		CircCCD C66	Circccdc66 regulates a subset of oncogenes, which control multiple pathological processes, including cell proliferation, migration, invasion, and anchorage-independent growth in CRC [77].
	Esopha geal Squam ous Cell	Has_circ_ 0067934	Has_circ_0067934 is upregulated and it accelerates malignant cell proliferation [78].

Carcinoma (ESCC)	Cir-ITCH	Cir-ITCH can inhibit ESCC proliferation by suppressing Wnt/-catenin pathway through sponging activity on miRNAs such as mich-7, mir-17 and mir-214 and thereby it enhances expression of ITCH [16].	
Hepato cellular Carcinoma (HCC)	CircZKSCAN1	CircZKSCAN1 inhibits HCC cell growth, migration, and invasion by blocking several signaling pathways [78].	
	Cdrlas	Mir-7 is a tumor suppressing ncRNA, which attenuates HCC proliferation and it decreases the risk of microvascular invasion (MVI) by suppressing its target gene PIK3CD and p70s6k expression. However, miR-7 activity is counteracted by the overexpression of cdrlas, which adsorbs miR-7 [79].	
	Hsa_circ_0005075	Hsa_circ_0005075 participates in cell adhesion during HCC development [80].	
	Hsa_circ_0004018; hsa_circ_0001649; circ-ITCH	They are significantly downregulated in HCC [81-83].	
	Hsa_circ_0085154	It is downregulated by androgen receptor (AR) dependent activation of ADAR1 expression in both HCC cells and malignant tissue [84].	
Cervical Cancer	Cdrlas	FAK promotes the proliferation, invasion and migration of cervical cancer cells and that exacerbates the progression of the disease. CDRlas promotes FAK expression by inhibiting miR-7, which targets FAK	

		[85].
	circRNA-000284	It promotes cell proliferation and invasion in cervical cancer [86].
Breast Cancer	Circ-Amotl1	Many circRNA are differentially expressed in breast cancer [87]. Hippo signaling promotes breast cancer progression by upregulating the expression of AMOTL1 and favouring metastasis [88]. Circ-Amotl1 interacts with c-myc and translocates to nucleus [89].
Human oral squamous cell carcinomas (OSCC).	Circrna_100290	It acts as a competing endogenous RNA to regulate CDK6 expression through sponging up miR-29b family members [90].
Lung adenocarcinoma (LAC)	Hsa_circ_0013958	It promotes cell proliferation and invasion. It inhibits cell apoptosis [91].
Bladder carcinoma	CircTCF25	It downregulates mir-103a-3p and mir-107, and increases the expression of CDK6. It promotes cell proliferation and migration [92].
	CircPTK2	It promotes proliferation and migration of bladder cancer cells [93].

HRCR: heart-related circRNA; Canril: circular antisense non-coding RNA at the INK4 locus; MFACR: mitochondrial fission and apoptosis-related circRNA;

Table 3 (on next page)

The Database for CircRNA Research

Every database present has its own sphere of competence, only the perfect combination of various database can provide information accurate.

1

Table 3 The Database for CircRNA Research

Tool Name	The latest version	URL	Remarks
circlncRNAnet [102]	May 2017	http://app.cgu.edu.tw/circlnc /	It aims to broaden the understanding of ncRNA candidates by testing in silico several hypotheses of ncRNA-based functions, on the basis of large-scale RNA-seq data.
starBase v2. 0[103]	December 2013	http://starbase. sysu. edu. cn/	Including microRNA, mRNA, lncRNA and other RNA information. It is a most useful tool for detecting miRNA-circRNA interaction. If there is need for retrieve all circRNAs in the genome, circRNABase is useful.
circBase[104]	December 2015	http://www. circbase. org/	Thousands of circular RNAs (circRNA) are annotated from eukaryotic cells.
circ2Traits[105]	December 2013	http://gyanxet-beta. com/circdb	Provides more information about the genomic positions of circRNA and cirRNA-associated diseases.
nc2Cancer[106]		http://www. Bioinfo. Tsinghua. Edu. cn/nc2Cance	
CircNet[107]	December 2015	http://circnet. mbc. nctu. edu. tw/	A database of circular RNAs derived from transcriptome sequencing data and this tool has most CircRNA annotation, in particularly from human.
deepBase v2. 0[108]	November 2015	http://biocenter. sysu. edu. cn/deepBase/	This database is a platform for annotation and discovery of small (microRNAs, siRNAs, piRNAs.) and long ncRNAs from next generation sequencing data.
CircInteracto me[109]	December 2015	http://circinteractome. nia. nih. gov/	This database can be used only to match the circRNA with relevant RNA binding proteins.
TSCD[110]	August 2016	http://gb. whu. edu. cn/TSCD/	It is useful for characterizing tissue-specific circRNA in human and mouse genomes.
CIRCpedia[11 1]	January 2015	http://www. picb. ac. cn/rnomics/circpedia/	This database contains reverse splicing and variable splicing sites of circRNA from 39 individuals and mouse samples.
circRNADb[11		http://reprod. njmu. edu.	It contains a record for more

| 2] | cn/circrnadb | than 30,000 exons with cyclic RNA nature in human genome |

2   Every database present has its own sphere of competence, only the perfect combination
3   of various database can provide information accurate.

4

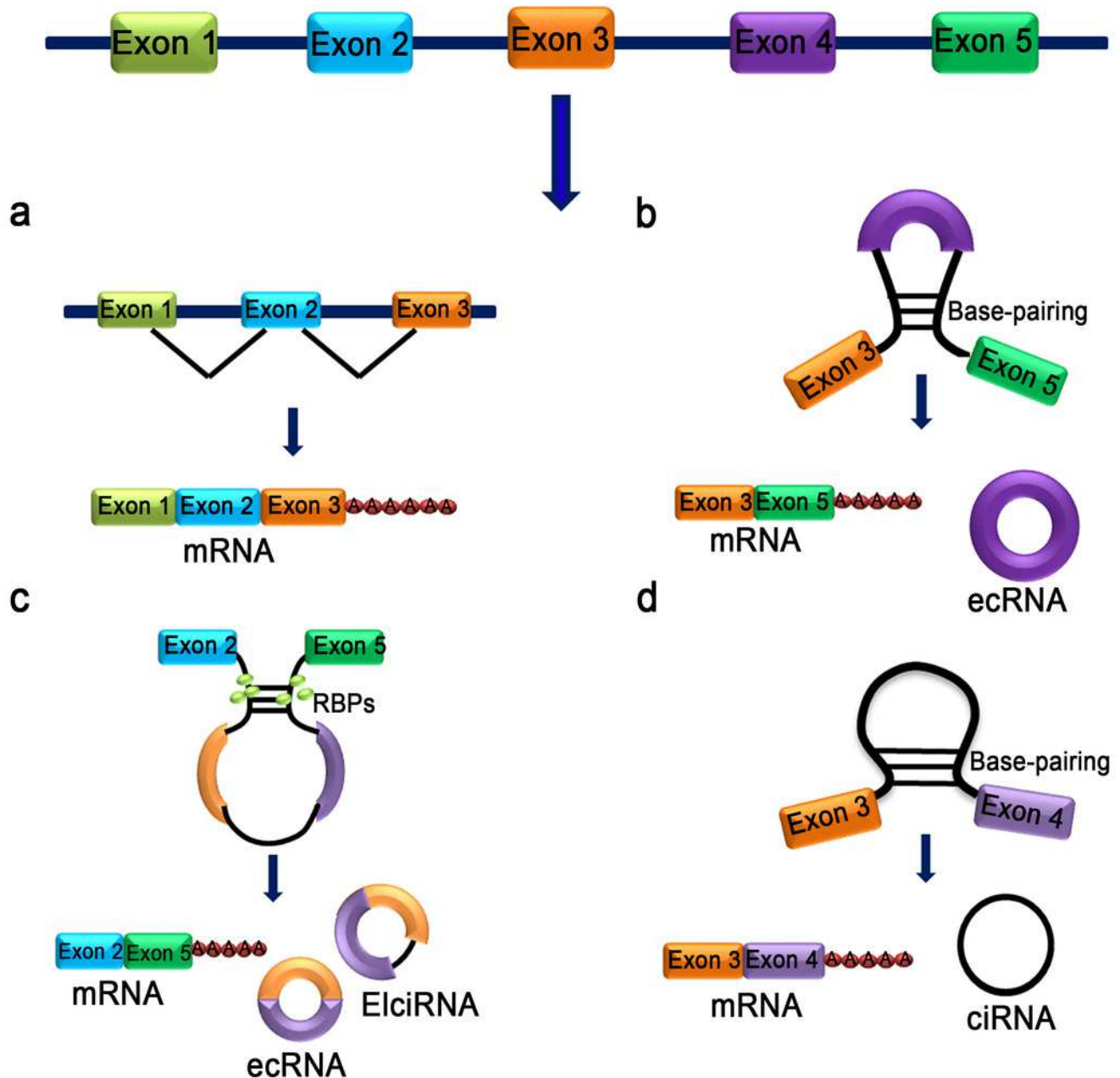# Figure 1

Characteristics of Different Types of CircRNA

Figure 2

Two Different Models of Exon Circularization of CircRNA

'(a) intron-pairing-driven circularization : d uring the formation of circRNA, an intron reverse complementary motif comprising GU-rich and C-rich elements is the key component to facilitate cyclization. ⬚   b) lariat-driven circularization : t he formation of circRNA is facilitated by the lariat structure. The complementary ALU flanking elements which is repeated in the intron region compete for classical linear RNA splicing and the circularization is accelerated by reverse complementarity [28] . '
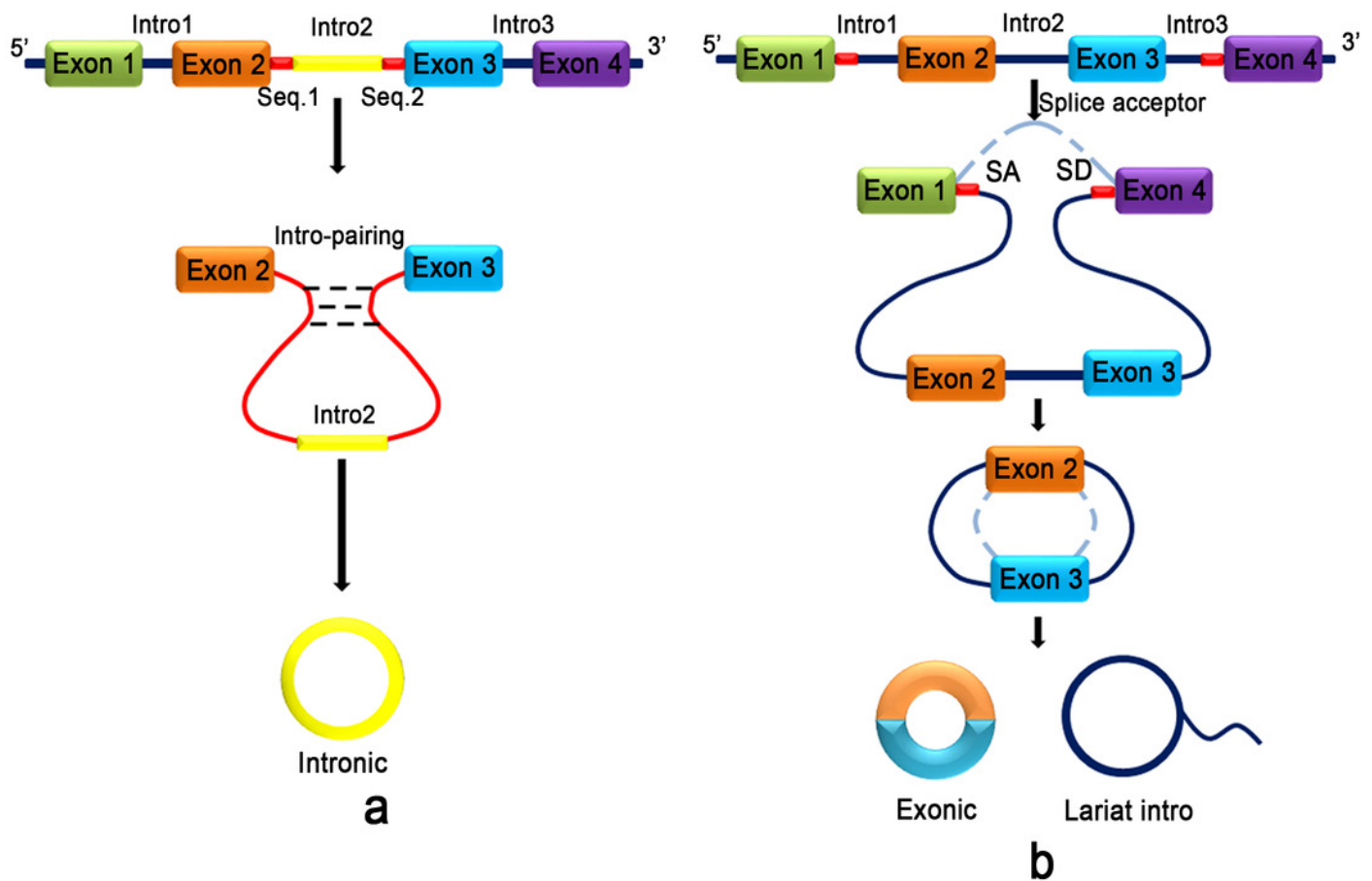
Figure 3

The five main functions of the circRNA

'(a) miRNA sponging activity: circRNA binds with miRNA and affecting the miRNA dependent target gene suppression.(b) Regulating selective splicing or transcription: Stable circRNA and EIciRNAs are located in the nucleus, where they bind to RNA polymerase and promoting transcription; circRNA competes with pre-mRNA splicing to reduce the level of linear mRNA and excludes specificity from pre-mRNA by changing the composition of processed mRNA. (c) Interaction with RBPs: circRNA binds with RBPs and ribonucleoprotein complexes and interfere with their functions . As a single circRNA can bind with multiple units of RBPs, they serve as stores of RBPs. (d)Rolling Circle Translation: Some circRNA can be translated into proteins by means of a roll loop amplification mechanism [28]. (e) Generation of Pseudogenes: Some circRNA are reverse transcribed into cDNA and integrated into the genome, however, the mechanism of integration is not yet clear. '
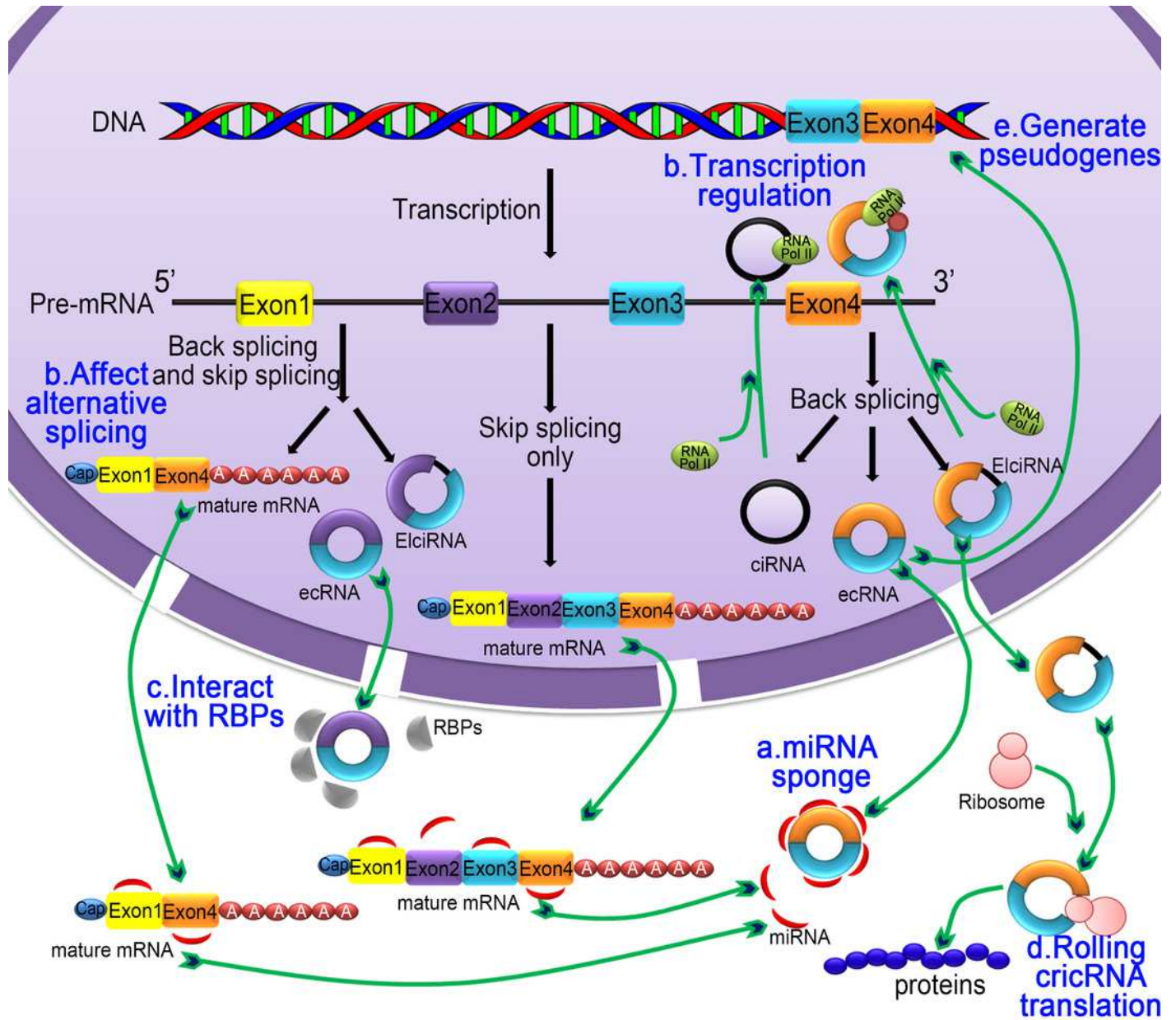
Figure 4

The Difference between Linear RNA and CircRNA primerdesign

'(a) FW is a forward primer with B chain as template. The base sequence of synthesis is the original sequence of A; RV is a reverse primer with A chain as template, and the base sequence of synthesis is the original sequence of B, The sequence between FW and RV is high. (b) Need to reverse the original primers: The synthetic primers are FW' and RV', where FW' is the reverse complementary sequence of the RV primer, RV' is the reverse complementary sequence of FW primer. '