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Emerging Roles and Context of Circular RNAs

Amaresh C Panda, Ioannis Grammatikakis, Rachel Munk, Myriam Gorospe, and Kotb Abdelmohsen*

Laboratory of Genetics, National Institute on Aging, National Institutes of Health, Baltimore, Maryland 21224, USA

Abstract

Circular RNAs (CircRNAs) represent a large class of noncoding RNAs (ncRNAs) that have recently emerged as regulators of gene expression. They have been shown to suppress microRNAs, thereby increasing the translation and stability of targets of such microRNAs. In this review, we discuss the emerging functions of circRNAs, including RNA transcription, splicing, turnover, and translation. We also discuss other possible facets of circRNAs that can influence their function depending on the cell context, such as circRNA abundance, subcellular localization, interacting partners (RNA, DNA, and proteins), dynamic changes in interactions following stimulation, and potential circRNA translation. The ensuing changes in gene expression patterns elicited by circRNAs are proposed to drive key cellular processes, such as cell proliferation, differentiation and survival, that govern health and disease.

Keywords

Ribonucleoprotein complexes; post-transcriptional gene regulation; transcriptome; mRNA stability; translation; decoy; splicing

INTRODUCTION

Circular RNAs (circRNAs) comprise a family of noncoding RNAs (ncRNAs) that have drawn intense interest in the last few years. Although they were first discovered in 1979 by electron microscopy, they were thought to be byproducts of splicing and did not receive much attention due to their low abundance and lack of known functions^{1, 2}. However, recent advances in RNA sequencing, quantitative PCR, and computational analysis revealed that circRNAs constitute a vastly abundant and heterogeneous class of RNAs, often expressed in a tissue-specific manner^{3–8}. Given their long half-life, circRNAs are emerging as critical post-transcriptional regulators of gene expression by binding microRNAs and buffering their repression of mRNA targets^{9, 10}. However, they may influence gene expression at other levels, such as transcription and splicing, as discussed below.

*Correspondence: Laboratory of Genetics, National Institute on Aging, National Institutes of Health, Biomedical Research Center, 251 Bayview Blvd., Baltimore, MD 21224, USA, Tel: 410-558-8589; Fax: 410-558-8331, abdelmohsenk@grc.nia.nih.gov.

CONFLICT OF INTEREST

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The biogenesis of circRNAs is not fully understood, but most circRNAs are believed to arise from the canonical spliceosomal machinery via head-to-tail backsplicing^{11–13}. About 14% of actively transcribed genes can produce circRNAs in fibroblasts⁵. A substantial fraction of spliced transcripts generate circRNAs, suggesting that RNA circularization is a conventional cellular feature^{14, 15}. In addition, more than one type of circular RNA containing exon(s), intron(s), or both can be produced from a single gene¹⁶. Recent studies and reviews have covered several aspects of circRNAs metabolism, including biogenesis, expression, regulation, and function^{14, 15, 17, 18}. Here, we will briefly discuss their known functions, and focus the discussion on considerations that are essential for elucidating the functions of circRNAs on key cellular processes. For instance, circRNAs may bind transcription factors and RNA-binding proteins forming ribonucleoprotein complexes with specific functions. CircRNAs may also form RNA-RNA complexes with long noncoding RNAs (lncRNAs) and mRNAs. These hybrid RNA complexes may alter the functions or the stability of both RNA molecules, while circRNA-mRNA complexes may alter mRNA stability or translation. The context in which circRNAs are found, including circRNA concentration, subcellular distribution, and dynamic interaction with molecules, will be proposed and discussed.

Biogenesis and types of circRNAs

Circularization of RNAs through backsplicing occurs by covalent end-joining of the 5' and 3' ends of the spliced RNA⁵. Another mechanism which has been proposed for biogenesis is through an exon-containing lariat precursor; in this case, an exon-skipping event creates a lariat containing an exon and internal splicing of the lariat removes the intronic sequence and produces a circRNA¹⁹. Despite low circRNA splicing efficiency, circRNA synthesis correlates with high transcription of the nascent mRNAs^{13, 20}. Long introns may play a role in backsplicing since they usually contain complementary inverted repeats such as Alu sequences that form double-stranded RNA (dsRNA) structures and theoretically bring the splice sites in close proximity^{5, 21, 22}. Along with RNA cis-elements that act on RNA circularization, trans-acting RBPs have also been found to regulate circRNA biogenesis. For instance, the RNA-editing enzyme adenosine deaminase acting on RNA-1 (ADAR1) is able to melt the dsRNA hairpin structure when inverted complementary sequences are present in the flanking introns of circularized exons²³ and thus inhibits circRNA biogenesis⁴. Since ADAR1 also functions as a dsRNA-binding protein²⁴, it may regulate circularization non-enzymatically. Two splicing regulators were recently identified as positive regulators of circRNA biogenesis, the proteins muscleblind (MBL) and quaking I 5 (QKI5)²⁵. MBL regulates circularization of exons derived from its own gene when bound to conserved binding sequences on both the exonic and flanking intronic sequences of *MBL* pre-mRNA. Further studies showed that addition of MBL binding sequences into a minigene was sufficient to induce circRNA biogenesis. In the same study, circRNA splicing was found to compete with the canonical linear splice sites²⁰. QKI5 is another RBP and splicing regulator which has been shown to regulate circRNA biogenesis. QKI5 is upregulated during endothelial-to-mesenchymal transition, indicating that circRNA biogenesis could be cell type-specific. Similar to MBL, QKI5 binding sites in intronic sequences flanking the circularized exons regulate circularization²⁵. Furthermore, in *Drosophila* it has been proposed that SR proteins and hnRNPs play a role in the formation of specific circRNAs²⁶.

One of the first circRNAs discovered comprises a single exon of the mouse sex-determining gene, *SRY*²⁷. CircRNAs may encompass just one or multiple exons which indicates the potential for alternatively spliced isoforms^{3, 22, 28}. They may also consist of intronic RNA sequences which are formed when intronic lariats fail to debranch. Another subset of circRNAs exists which contains both exons and introns (known as exon-intron-circRNAs or EIciRNAs)²⁹. The class of circular intronic RNAs (ciRNAs) is derived from lariat introns during canonical splicing; failure to debranch at the branch point site and trimming of the lariat tail leads to formation of stable ciRNA¹⁵. CircRNAs have been reviewed in detail, focusing on their biogenesis, classification, and possible role in diseases, and have been catalogued in databases (Fig. 1)^{18, 19, 30–32}. In this review, we will highlight these aspects, but will concentrate our discussion on new potential functions and considerations of these regulatory RNAs.

REPORTED circRNA FUNCTIONS

miRNA sponging

There are accumulating examples of circRNAs acting as miRNA sponges, thereby influencing the post-transcriptional actions of miRNAs as suppressors of the translation and/or stability of target mRNAs³³. The circRNA *CiRS-7* bears more than 70 miR-7 binding sites and thus acts as a miR-7 antagonist¹⁰, in turn limiting the impact of miR-7 on target mRNAs. Indeed, *CiRS-7* promoted myocardial infarction by sponging miR-7a and thus controlled the expression of the miR-7a targets *PARP* and *SP1* mRNA in myocardial cells³⁴. Sex-determining region Y (*SRY*), another circRNA expressed in murine testis, encompasses 16 binding sites for miR-138⁹, a microRNA implicated in several physiologic and pathologic processes.³⁵ *Cir-ITCH* was reported to sponge miR-7, miR-17, and miR-214, leading to the upregulation of *ITCH* (Itchy E3 ubiquitin protein ligase) and inhibition of WNT signaling in esophageal squamous cell carcinoma³⁶. *CircHIPK3*, generated from the second exon of *HIPK3* (homeodomain-interacting protein kinase 3) mRNA, can sponge miR-124 and other miRNAs; accordingly, silencing *circHIPK3* reduced cell growth, suggesting a role of *circHIPK3* in cell proliferation through changes in the availability of miR-124 to target mRNAs³⁷. Several circRNAs generated from cattle casein (*CSN*) genes, which are highly expressed in bovine mammary gland, can sponge microRNAs in the miR-2284 family, which target *CSN1S1* and *CSN2* mRNAs³⁸. Taken together, these reports suggest that the general ability of circRNAs to sponge miRNAs could be an internal cellular mechanism used to fine-tune miRNA actions (Table 1).

Transcription

Recently, circRNAs were shown to regulate transcription. Exon-intron circRNAs (EIciRNAs) in the nucleus were found to associate with RNA polymerase II and modify its transcriptional activity²⁹. For instance, EIciEIF3J interacts with the small nuclear ribonucleoprotein (snRNP) U1 and the promoter EIF3J to enhance EIF3J transcription²⁹. CircRNA *Cdr1as* (also known as *CiRS-7*) was found to regulate insulin transcription by sponging miR-7 and its targets³⁹, although the exact mechanism is not yet known (Table 1). Future studies will likely reveal other examples of transcriptional regulation by circRNAs.

Splicing

The impact of circRNAs on splicing is just emerging. Recently, the possibility that circRNA biogenesis is competing with splicing has begun to develop. The second exon of the *MBL* pre-mRNA mentioned above can circularize to form circMbl with flanking introns that strongly bind to MBL. CircMbl-MBL interaction modulates the splicing activity of MBL and regulates *MBL* pre-mRNA splicing by competing with the canonical splicing machinery (Table 1)²⁰. As our understanding of circRNAs continues to grow, additional examples of circRNAs influencing splicing events are also likely to arise.

Protein Decoy

Protein decoy or antagonist is another rising function of circRNAs. For instance, circ-Foxo3 is downregulated in cancer cells and is associated with cell cycle progression. Circ-Foxo3 binds cyclin-dependent kinase 2 (CDK2) and p21 (CDKN1A) forming an RNA-protein complex that disrupts the interactions of CDK2 with cyclins A and E, required for cell cycle progression (Table 1).⁴⁰ Circ-Foxo3 also interacts with proteins ID1, E2F1, FAK, and HIF-1 α (HIF1A) retaining them in the cytoplasm and thus promoting cardiac senescence⁴¹. These findings indicate that circRNAs may function as decoys that modify the cellular destination and/or function of bound factors.

CONSIDERATIONS FOR FUTURE circRNA ANALYSIS

Determine abundance and localization

One of the critical issues to consider regarding circRNA function is their abundance compared to their linear RNA counterparts (mRNAs or lncRNAs)^{5, 8}. Although generally circRNAs are less abundant than linear RNAs, in some instances circRNAs are in higher concentration. CircRNAs are usually more stable due to the lack of free ends⁴², but may be downregulated in certain disease conditions, like colorectal cancer⁴³, sporadic Alzheimer's disease⁴⁴ (as shown for ciRS-7), and during physiologic changes such as skeletal muscle aging in monkey⁷. Thus, it is imperative to determine the underlying mechanisms that govern circRNA levels under various conditions in different tissues. A related major consideration for circRNA function is their localization. It has been shown that exonic circRNAs are predominantly cytoplasmic⁵, but it will be essential to measure if intronic circRNAs and exon-intronic circRNAs are nuclear, cytoplasmic, or both. This knowledge will set the stage for understanding circRNA function and possibly for devising circRNA-based therapeutic approaches.

Test potential interplay of circRNAs with mRNAs, ncRNAs, and proteins

The association of circRNAs with miRNAs can impact upon mRNA translation and/or stability. In addition, circRNAs may interact directly with other RNA molecules such as mRNAs and lncRNAs. These interactions may influence the stability, translation, and localization of mRNAs and lncRNAs. For example, since translation efficiency is higher when an mRNA forms a loop⁴⁵, it is conceivable that circRNAs pairing with the 5' and 3' ends of target transcripts bring the two ends close enough to form a loop influencing mRNA translation. In addition, given that circRNAs may also interact with RBPs and even act as

RBP sponges, the interaction of circRNAs with HuR, KSRBP, TTP, AUF1, and other potent regulatory RBPs could affect the fate of their respective target mRNAs^{46, 47}, in turn affecting their splicing, transport, storage, turnover, and translation. Reciprocally, binding of RBPs to mRNAs may impact upon circRNA processing, function, abundance, and/or subcellular localization. It is important to note that despite the fact that circRNAs are considered to be highly stable, their turnover has not been studied after altering the levels of interacting RBPs. Similarly, circRNAs interacting with transcription factors (TFs) may form circRNA-TF complexes that influence TF translocation into the nucleus and/or transcriptional activity. Such influence has been observed in lncRNAs such as *GAS5*, which acts as a decoy for the TF glucocorticoid receptor, and prevents it from binding to DNA to elicit transcriptional activation⁴⁸. As mentioned above, circ-Foxo3 is considered to be a decoy for TFs. Future analysis of the dynamic associations of circRNAs with RBPs, TFs, and RNAs will undoubtedly uncover rich and versatile mechanisms by which circRNAs control gene expression programs (Fig. 2).

Evaluate dynamic interactions of circRNAs under basal and stimulated conditions

While the association of circRNAs with other cellular factors (RNAs or proteins) can occur in unstimulated conditions such as those mentioned here, the expectation is that circRNA functions can be altered in response to changing conditions in the cell. Exposure to various stimuli or pathological conditions could alter circRNA interactions with DNA, RNA or protein, due to changes in the abundance, type, structure, localization, or function of the interacting factors. In turn, the new circRNA-associated factors could impact upon the components of the RNP and the circRNA in similar ways, by changing their abundance, localization, function, structure, and so on. In particular, regarding structure, it is important to note that changes in circRNA folding may expose or hide binding sites of other RNAs or proteins, and thus influence circRNA function. As circRNAs are generated in the nucleus during splicing, it is expected that many of the intron-containing circRNAs are retained in the nucleus and may influence the splicing of pre-RNA counterparts by competing with splicing regulators.

Potential circRNA-DNA interaction

During DNA replication, two strands of the genomic DNA separate to form single-stranded DNA. CircRNAs in the nucleus may interact with the opposite strand of its genomic DNA via sequence complementarity which may form a DNA-RNA triple helix affecting DNA replication. Linear noncoding RNAs residing in the nucleus were found to interact with DNA forming RNA-DNA duplexes and triplexes in association with proteins to control gene expression. For instance, pRNA (promoter-associated RNA) forms a triplex with the binding site of ITTF1 and simultaneously recruits DNMT3b to repress rRNA expression, suggesting a transcription-associated interaction with the genomic locus⁴⁹. It has been suggested that lncRNA *ANRASSF1* interacts with DNA forming RNA/DNA hybrid at the transcription start site that facilitates the recruitment of the chromatin-modifying PRC2 complex leading to a specific reduction in *RASSF1A* transcriptional activity⁵⁰. In addition, mapping of the lncRNA *HOTAIR* identified enriched DNA-interacting sequences⁵¹. These and other examples have been reviewed^{52, 53}. In a manner akin to linear noncoding RNAs, the

interactions between DNA and nucleus-residing circRNAs, particularly those comprising intronic sequences, is another area that warrants exploration.

Assess possible circRNA translation

General translation of mRNAs begins when the translation machinery recognizes the 5' UTR cap, scans for the start codon and generates a polypeptide until it encounters a stop codon⁵⁴. Cap-independent translation can also occur, and requires an internal ribosomal entry site (IRES).⁵⁵ Interestingly, circular mRNAs have been engineered to generate large proteins *in vitro* by removing the stop codon⁵⁶. A limited screen of circRNAs indicated that they are not associated with polyribosomes, suggesting the possibility that circRNAs are not translated⁵. However, circRNAs may not require large polyribosomes, and instead just a few ribosomes can generate small proteins or micropeptides. In this regard, a micropeptide was found to be encoded by a linear noncoding RNA that regulates muscle performance⁵⁷, and the viral circRNA CCC (Covalently Closed Circular, 220-nt long) was found to be translated into a 16-kDa protein in infected rice plants⁵⁸. Bioinformatic analysis uncovered several circRNAs that encompass IRESs, indicating that they could putatively be translated into small proteins or micropeptides³². An *in vitro* synthesized circRNA with an IRES was shown to bind the 40S ribosomal subunit and initiated translation⁵⁹. Another report suggested translation of an artificial circRNA with a GFP open reading frame in *Escherichia coli*⁵⁶, and a recent study suggested that mRNAs with N6-methyladenosine (m6A) modification in the 5' UTR can be translated in a cap-independent manner through m6A interaction with the eukaryotic initiation factor 3 (eIF3)^{60, 61}. Thus, it will be interesting to investigate whether IRESs and/or m6A modification occur in circRNAs, and if they have an impact on cap-independent circRNA translation. We hypothesize that some circRNAs that contain short ORFs, like linear lncRNAs, could be translated to generate small proteins, micropeptides, and even truncated or chimeric proteins. It will be particularly important to establish more comprehensively whether subsets of circRNAs associate with polysomes and might be translated.

CLOSING REMARKS

Intense efforts are underway to elucidate the functions of circular RNAs. Some examples of circRNAs affecting gene expression are already becoming apparent. Given that they can bind microRNAs and proteins involved in transcription and splicing, circRNAs are widely believed to influence mRNA metabolism on many levels (transcription, splicing, mRNA turnover, translation) (Fig. 2). Based on the same ability to bind nucleic acids and proteins, additional functions are hypothesized for circRNAs, such as their translation via IRES elements and interaction with DNA. However, as highlighted in this review, these interactions must be investigated in the context of (1) the levels of circRNAs and associated factors, which may change with development, cell stimuli, disease states, and other conditions; (2) the dynamic interactions among the circRNA-associated factors, which can compete, cooperate, and otherwise influence the composition of the circRNA molecular complex; and (3) the localization of the circRNA in distinct cellular locales (nucleus, cytosol, endoplasmic reticulum, mitochondria, extracellular vesicles, etc.), since specific sets of circRNA-interacting molecules reside in each subcellular compartment. The ongoing

efforts to elucidate circRNA function must include these considerations, as they will illuminate more fully the rich and versatile impact of circRNAs in physiology and pathology.

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References

1. Hsu MT, Coca-Prados M. Electron microscopic evidence for the circular form of RNA in the cytoplasm of eukaryotic cells. *Nature*. 1979; 280:339–340. [PubMed: 460409]
2. Nigro JM, Cho KR, Fearon ER, Kern SE, Ruppert JM, Oliner JD, Kinzler KW, Vogelstein B. Scrambled Exons. *Cell*. 1991; 64:607–613. [PubMed: 1991322]
3. Salzman J, Chen RE, Olsen MN, Wang PL, Brown PO. Cell-type specific features of circular RNA expression. *PLoS Genet*. 2013; 9:e1003777. [PubMed: 24039610]
4. Rybak-Wolf A, Stottmeister C, Glazar P, Jens M, Pino N, Giusti S, Hanan M, Behm M, Bartok O, Ashwal-Fluss R, et al. Circular RNAs in the Mammalian Brain Are Highly Abundant, Conserved, and Dynamically Expressed. *Mol Cell*. 2015; 58:870–885. [PubMed: 25921068]
5. Jeck WR, Sorrentino JA, Wang K, Slevin MK, Burd CE, Liu J, Marzluff WF, Sharpless NE. Circular RNAs are abundant, conserved, and associated with ALU repeats. *RNA*. 2013; 19:141–157. [PubMed: 23249747]
6. Glazar P, Papavasileiou P, Rajewsky N. circBase: a database for circular RNAs. *RNA*. 2014; 20:1666–1670. [PubMed: 25234927]
7. Abdelmohsen K, Panda AC, De S, Grammatikakis I, Kim J, Ding J, Noh JH, Kim KM, Mattison JA, de Cabo R, et al. Circular RNAs in monkey muscle: age-dependent changes. *Aging (Albany NY)*. 2015; 7:903–910. [PubMed: 26546448]
8. Guo JU, Agarwal V, Guo H, Bartel DP. Expanded identification and characterization of mammalian circular RNAs. *Genome Biol*. 2014; 15:409. [PubMed: 25070500]
9. Hansen TB, Jensen TI, Clausen BH, Bramsen JB, Finsen B, Damgaard CK, Kjems J. Natural RNA circles function as efficient microRNA sponges. *Nature*. 2013; 495:384–388. [PubMed: 23446346]
10. Memczak S, Jens M, Elefsinioti A, Torti F, Krueger J, Rybak A, Maier L, Mackowiak SD, Gregersen LH, Munschauer M, et al. Circular RNAs are a large class of animal RNAs with regulatory potency. *Nature*. 2013; 495:333–338. [PubMed: 23446348]
11. Wilusz JE, Sharp PA. Molecular biology. A circuitous route to noncoding RNA. *Science*. 2013; 340:440–441. [PubMed: 23620042]
12. Jeck WR, Sharpless NE. Detecting and characterizing circular RNAs. *Nat Biotechnol*. 2014; 32:453–461. [PubMed: 24811520]
13. Zhang Y, Xue W, Li X, Zhang J, Chen S, Zhang JL, Yang L, Chen LL. The Biogenesis of Nascent Circular RNAs. *Cell Rep*. 2016; 15:611–624. [PubMed: 27068474]
14. Salzman J. Circular RNA Expression: Its Potential Regulation and Function. *Trends Genet*. 2016; 32:309–316. [PubMed: 27050930]
15. Chen LL. The biogenesis and emerging roles of circular RNAs. *Nat Rev Mol Cell Biol*. 2016; 17:205–211. [PubMed: 26908011]
16. Vicens Q, Westhof E. Biogenesis of Circular RNAs. *Cell*. 2014; 159:13–14. [PubMed: 25259915]
17. Li J, Yang J, Zhou P, Le Y, Zhou C, Wang S, Xu D, Lin HK, Gong Z. Circular RNAs in cancer: novel insights into origins, properties, functions and implications. *Am J Cancer Res*. 2015; 5:472–480. [PubMed: 25973291]
18. Shao Y, Chen Y. Roles of Circular RNAs in Neurologic Disease. *Front Mol Neurosci*. 2016; 9:25. [PubMed: 27147959]
19. Barrett SP, Wang PL, Salzman J. Circular RNA biogenesis can proceed through an exon-containing lariat precursor. *Elife*. 2015; 4:e07540. [PubMed: 26057830]

20. Ashwal-Fluss R, Meyer M, Pamudurti NR, Ivanov A, Bartok O, Hanan M, Evtantal N, Memczak S, Rajewsky N, Kadener S. circRNA biogenesis competes with pre-mRNA splicing. *Mol Cell*. 2014; 56:55–66. [PubMed: 25242144]
21. Liang D, Wilusz JE. Short intronic repeat sequences facilitate circular RNA production. *Genes Dev*. 2014; 28:2233–2247. [PubMed: 25281217]
22. Zhang XO, Wang HB, Zhang Y, Lu X, Chen LL, Yang L. Complementary sequence-mediated exon circularization. *Cell*. 2014; 159:134–147. [PubMed: 25242744]
23. Ivanov A, Memczak S, Wyler E, Torti F, Porath HT, Orejuela MR, Piechotta M, Levanon EY, Landthaler M, Dieterich C, et al. Analysis of intron sequences reveals hallmarks of circular RNA biogenesis in animals. *Cell Rep*. 2015; 10:170–177. [PubMed: 25558066]
24. Chen T, Xiang JF, Zhu S, Chen S, Yin QF, Zhang XO, Zhang J, Feng H, Dong R, Li XJ, et al. ADAR1 is required for differentiation and neural induction by regulating microRNA processing in a catalytically independent manner. *Cell Res*. 2015; 25:459–476. [PubMed: 25708366]
25. Conn SJ, Pillman KA, Toubia J, Conn VM, Salamanidis M, Phillips CA, Roslan S, Schreiber AW, Gregory PA, Goodall GJ. The RNA binding protein quaking regulates formation of circRNAs. *Cell*. 2015; 160:1125–1134. [PubMed: 25768908]
26. Kramer MC, Liang D, Tatomer DC, Gold B, March ZM, Cherry S, Wilusz JE. Combinatorial control of Drosophila circular RNA expression by intronic repeats, hnRNPs, and SR proteins. *Genes Dev*. 2015; 29:2168–2182. [PubMed: 26450910]
27. Capel B, Swain A, Nicolis S, Hacker A, Walter M, Koopman P, Goodfellow P, Lovell-Badge R. Circular transcripts of the testis-determining gene Sry in adult mouse testis. *Cell*. 1993; 73:1019–1030. [PubMed: 7684656]
28. Westholm JO, Miura P, Olson S, Shenker S, Joseph B, Sanfilippo P, Celniker SE, Graveley BR, Lai EC. Genome-wide analysis of drosophila circular RNAs reveals their structural and sequence properties and age-dependent neural accumulation. *Cell Rep*. 2014; 9:1966–1980. [PubMed: 25544350]
29. Li Z, Huang C, Bao C, Chen L, Lin M, Wang X, Zhong G, Yu B, Hu W, Dai L, et al. Exon-intron circular RNAs regulate transcription in the nucleus. *Nat Struct Mol Biol*. 2015; 22:256–264. [PubMed: 25664725]
30. Wilusz JE. Repetitive elements regulate circular RNA biogenesis. *Mob Genet Elements*. 2015; 5:1–7.
31. Hansen TB, Veno MT, Damgaard CK, Kjems J. Comparison of circular RNA prediction tools. *Nucleic Acids Res*. 2016; 44:e58. [PubMed: 26657634]
32. Dudekula DB, Panda AC, Grammatikakis I, De S, Abdelmohsen K, Gorospe M. CircInteractome: A web tool for exploring circular RNAs and their interacting proteins and microRNAs. *RNA Biol*. 2016; 13:34–42. [PubMed: 26669964]
33. Valinezhad Orang A, Safaralizadeh R, Kazemzadeh-Bavili M. Mechanisms of miRNA-Mediated Gene Regulation from Common Downregulation to mRNA-Specific Upregulation. *Int J Genomics*. 2014; 2014:970607. [PubMed: 25180174]
34. Geng HH, Li R, Su YM, Xiao J, Pan M, Cai XX, Ji XP. The Circular RNA Cdr1as Promotes Myocardial Infarction by Mediating the Regulation of miR-7a on Its Target Genes Expression. *PLoS One*. 2016; 11:e0151753. [PubMed: 26998750]
35. Han L, Zhang G, Zhang N, Li H, Liu Y, Fu A, Zheng Y. Prognostic potential of microRNA-138 and its target mRNA PDK1 in sera for patients with non-small cell lung cancer. *Med Oncol*. 2014; 31:129. [PubMed: 25064732]
36. Li F, Zhang L, Li W, Deng J, Zheng J, An M, Lu J, Zhou Y. Circular RNA ITCH has inhibitory effect on ESCC by suppressing the Wnt/beta-catenin pathway. *Oncotarget*. 2015; 6:6001–6013. [PubMed: 25749389]
37. Zheng Q, Bao C, Guo W, Li S, Chen J, Chen B, Luo Y, Lyu D, Li Y, Shi G, et al. Circular RNA profiling reveals an abundant circHIPK3 that regulates cell growth by sponging multiple miRNAs. *Nat Commun*. 2016; 7:11215. [PubMed: 27050392]
38. Zhang C, Wu H, Wang Y, Zhu S, Liu J, Fang X, Chen H. Circular RNA of cattle casein genes are highly expressed in bovine mammary gland. *J Dairy Sci*. 2016

39. Xu H, Guo S, Li W, Yu P. The circular RNA Cdr1as, via miR-7 and its targets, regulates insulin transcription and secretion in islet cells. *Sci Rep.* 2015; 5:12453. [PubMed: 26211738]
40. Du WW, Yang W, Liu E, Yang Z, Dhaliwal P, Yang BB. Foxo3 circular RNA retards cell cycle progression via forming ternary complexes with p21 and CDK2. *Nucleic Acids Res.* 2016; 44:2846–2858. [PubMed: 26861625]
41. Du WW, Yang W, Chen Y, Wu ZK, Foster FS, Yang Z, Li X, Yang BB. Foxo3 circular RNA promotes cardiac senescence by modulating multiple factors associated with stress and senescence responses. *Eur Heart J.* 2016
42. Enuoka Y, Lauriola M, Feldman ME, Sas-Chen A, Ulitsky I, Yarden Y. Circular RNAs are long-lived and display only minimal early alterations in response to a growth factor. *Nucleic Acids Res.* 2016; 44:1370–1383. [PubMed: 26657629]
43. Bachmayr-Heyda A, Reiner AT, Auer K, Sukhbaatar N, Aust S, Bachleitner-Hofmann T, Mesteri I, Grunt TW, Zeillinger R, Pils D. Correlation of circular RNA abundance with proliferation--exemplified with colorectal and ovarian cancer, idiopathic lung fibrosis, and normal human tissues. *Sci Rep.* 2015; 5:8057. [PubMed: 25624062]
44. Lukiw WJ. Circular RNA (circRNA) in Alzheimer's disease (AD). *Front Genet.* 2013; 4:307. [PubMed: 24427167]
45. Kahvejian A, Roy G, Sonenberg N. The mRNA closed-loop model: the function of PABP and PABP-interacting proteins in mRNA translation. *Cold Spring Harb Symp Quant Biol.* 2001; 66:293–300. [PubMed: 12762031]
46. Abdelmohsen K, Gorospe M. Posttranscriptional regulation of cancer traits by HuR. *Wiley Interdiscip Rev RNA.* 2010; 1:214–229. [PubMed: 21935886]
47. White EJ, Brewer G, Wilson GM. Post-transcriptional control of gene expression by AUF1: mechanisms, physiological targets, and regulation. *Biochim Biophys Acta.* 2013; 1829:680–688. [PubMed: 23246978]
48. Williams GT, Mourtada-Maarabouni M, Farzaneh F. A critical role for non-coding RNA GAS5 in growth arrest and rapamycin inhibition in human T-lymphocytes. *Biochem Soc Trans.* 2011; 39:482–486. [PubMed: 21428924]
49. Schmitz KM, Mayer C, Postepska A, Grummt I. Interaction of noncoding RNA with the rDNA promoter mediates recruitment of DNMT3b and silencing of rRNA genes. *Genes Dev.* 2010; 24:2264–2269. [PubMed: 20952535]
50. Beckedorff FC, Ayupe AC, Crocci-Souza R, Amaral MS, Nakaya HI, Soltys DT, Menck CF, Reis EM, Verjovski-Almeida S. The intronic long noncoding RNA ANRASSF1 recruits PRC2 to the RASSF1A promoter, reducing the expression of RASSF1A and increasing cell proliferation. *PLoS Genet.* 2013; 9:e1003705. [PubMed: 23990798]
51. Chu C, Qu K, Zhong FL, Artandi SE, Chang HY. Genomic maps of long noncoding RNA occupancy reveal principles of RNA-chromatin interactions. *Mol Cell.* 2011; 44:667–678. [PubMed: 21963238]
52. Villegas VE, Zaphiropoulos PG. Neighboring gene regulation by antisense long non-coding RNAs. *Int J Mol Sci.* 2015; 16:3251–3266. [PubMed: 25654223]
53. Mercer TR, Mattick JS. Structure and function of long noncoding RNAs in epigenetic regulation. *Nat Struct Mol Biol.* 2013; 20:300–307. [PubMed: 23463315]
54. Hershey JW, Sonenberg N, Mathews MB. Principles of translational control: an overview. *Cold Spring Harb Perspect Biol.* 2012:4.
55. Pacheco A, Martinez-Salas E. Insights into the biology of IRES elements through riboproteomic approaches. *J Biomed Biotechnol.* 2010; 2010:458927. [PubMed: 20150968]
56. Perriman R, Ares M. Circular mRNA can direct translation of extremely long repeating-sequence proteins in vivo. *Rna-a Publication of the Rna Society.* 1998; 4:1047–1054.
57. Anderson DM, Anderson KM, Chang CL, Makarewich CA, Nelson BR, McAnally JR, Kasaragod P, Shelton JM, Liou J, Bassel-Duby R, et al. A Micropeptide Encoded by a Putative Long Noncoding RNA Regulates Muscle Performance. *Cell.* 2015; 160:595–606. [PubMed: 25640239]
58. AbouHaidar MG, Venkataraman S, Golshani A, Liu BL, Ahmad T. Novel coding, translation, and gene expression of a replicating covalently closed circular RNA of 220 nt. *Proc Natl Acad Sci U S A.* 2014; 111:14542–14547. [PubMed: 25253891]

59. Chen CY, Sarnow P. Initiation of protein synthesis by the eukaryotic translational apparatus on circular RNAs. *Science*. 1995; 268:415–417. [PubMed: 7536344]
60. Meyer KD, Patil DP, Zhou J, Zinoviev A, Skabkin MA, Elemento O, Pestova TV, Qian SB, Jaffrey SR. 5' UTR m(6)A Promotes Cap-Independent Translation. *Cell*. 2015; 163:999–1010. [PubMed: 26593424]
61. Zhou J, Wan J, Gao X, Zhang X, Jaffrey SR, Qian SB. Dynamic m(6)A mRNA methylation directs translational control of heat shock response. *Nature*. 2015; 526:591–594. [PubMed: 26458103]

Figure 1. Schematic representation of splicing events leading to the generation of circRNA
(A) The canonical splicing machinery conventionally generates normal mRNA. **(B)** Exonic circular RNA (circRNA) is generated through noncanonical splicing (backsplicing) through the unique ‘head-to tail’ joining of the 5′ splice site (5’ss, donor site) to an 3′ splice site (3’ss, acceptor site). RNA-binding proteins (RBP) or *trans*-acting factors can bridge two flanking introns close together. The introns are then removed to form a circRNA. **(C)** Reverse complementary sequences (purple arrows) in Intron1 and Intron3 can pair and bring the 5’ss of Exon3 close to 3’ss of Exon2, promote circularization of Exon2 and Exon3 with a retained intron, and form an Exon-Intron circRNA (EIciRNA). In addition, backsplicing in combination of canonical splicing may lead to the formation of circRNA with Exon2 and Exon3 only. **(D)** The circular intronic RNA (ciRNA) is derived from the lariat intron excised from pre-mRNA by canonical splicing machinery and depends on the presence of consensus RNA sequences (yellow bars) to avoid debranching of the lariat intron to form stable ciRNAs. The red and blue dotted lines indicate linear and head-to-tail backsplicing, respectively.

Figure 2. Emerging activities, proposed functions, and dynamic interactions of circRNAs
 (From *top clockwise*) CircRNAs may influence transcription factor (TF) function by influencing TF localization and/or activity. CircRNAs may also may also associate with traditional RNA-binding proteins (RBPs) or multi-RBP complexes and influence the fate of the circRNAs themselves (e.g., localization, stability), impact upon the mRNAs that the RBPs interact with (e.g., mRNA stability or translation), or perhaps serve as a platform for the assembly of multi-protein complexes. Partial interaction of circRNAs with mRNAs can similarly lead to altered mRNA turnover and/or translation. CircRNAs may also interact with single-stranded (as in the schematic) or double-stranded DNA forming double or triple helices, respectively, with potential impact on DNA metabolism (e.g., transcription, replication). The interaction of circRNAs with linear lncRNAs could directly affect lncRNA

functions (e.g., localization, folding, etc.) or impact upon the target molecules (e.g., RNAs or proteins) with which the lncRNA associates. The ability to sequester or 'sponge' microRNAs is another recognized function of circRNAs. As several circRNAs comprise exonic 5'UTR segments that bear internal ribosome entry sites (IRESs), circRNAs may also serve as templates for translation synthesis. Finally, circRNA interaction with splicing factors may affect splicing of pre-mRNAs.

Shaded text boxes, additional considerations critical for the study of circRNAs, including the aforementioned interactions with DNA in the nucleus and with the translation machinery via IRESs in the cytosol. CircRNA levels change as a function of development, in disease states, and in response to stress, immune, hormonal or other stimuli (*left*). CircRNA interactions with RNA, protein, and DNA may change depending on the cell context, and these interactions may be competitive, cooperative, sequential, etc. The localization of circRNA intracellularly and possibly even outside should be examined, as it impacts directly on its function.

Table 1

Reported circular RNA functions

CircRNA name	Parent gene	Function	References
<i>CIRS-7/CDR1as</i>	Cerebellar degeneration-related protein 1 (<i>Cdr1</i>) antisense transcript	miR-7 antagonist, myocardial infarction, insulin transcription	10, 34, 39
<i>circSRY</i>	Sex-determining region Y (<i>SRY</i>)	miR-138 sponging	9
<i>circ-ITCH</i>	Itchy E3 ubiquitin protein ligase (<i>ITCH</i>)	miR-7, miR-17, and miR-214 sponging; inhibition of WNT signaling in esophageal squamous cell carcinoma	36
<i>circHIPK3</i>	Homeodomain interacting protein kinase 3 (<i>HIPK3</i>)	miR-124 sponging, cell proliferation	37
<i>Cattle casein circRNAs</i>	Cattle casein genes <i>CSN1S1</i> , <i>CSN1S2</i> , <i>CSN2</i> , and <i>CSN3</i>	miR-2284 family sponging	38
<i>ElciEIF3J</i>	Eukaryotic translation initiation factor 3 subunit J (<i>EIF3J</i>)	Upregulates EIF3J transcription by interacting with small nuclear ribonucleoprotein (snRNP) U1 on <i>EIF3J</i> promoter	29
<i>circMbl</i>	Muscleblind (<i>Mbl</i>)	biogenesis interferes with canonical splicing	20
<i>Circ-Fox3</i>	Forkhead box 3 (<i>Fox3</i>)	protein decoy for CDK2, p21, ID1, E2F1, FAK, and HIF-1 α , cell cycle progression, cardiac cell senescence	40, 41