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Circular RNAs: Unraveling Their Role in Regulation, Development, and Disease

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Introduction to Non-Coding RNAs

The human genome comprises approximately 3 billion base pairs across 23 pairs of chromosomes, yet less than 2% of these sequences encode proteins (Julia di Iulio, 2018). The remaining majority transcribe into non-coding RNAs (ncRNAs), which do not translate into proteins but play pivotal roles in cellular regulation. These ncRNAs fall into two broad categories: small ncRNAs (<30 nucleotides) such as miRNA, siRNA, and piRNA, and long ncRNAs (>200 nucleotides).

Small non-coding RNAs (ncRNAs) regulate gene expression through mechanisms such as gene silencing and chromatin remodeling, playing crucial roles in cellular processes (Rederstorff & Huttenhofer, 2010; Carthew, 2009). Long non-coding RNAs (lncRNAs) are key regulators in development and disease, often acting as molecular decoys or sponges that sequester miRNAs and mRNAs, thereby modulating gene expression (Ma et al., 2015; Paci, 2014). The first bacterial ncRNA, micF, was identified in E. coli in 1980, marking an important milestone in our understanding of ncRNA function (Delihas, 2015). More recently, circular RNAs (circRNAs), a novel class of lncRNAs, have garnered increasing attention due to their emerging roles in gene regulation, the cellular response to stress, and the development of various diseases (Memczak, 2013). These circRNAs are unique in that they form covalently closed loops, making them resistant to exonuclease degradation, and they can function as regulators of transcription, splicing, and protein interaction.

Circular RNAs – Features and Functions

CircRNAs are a novel class of lncRNAs formed through a back-splicing mechanism where a downstream splice donor joins an upstream splice acceptor, creating a covalently closed loop (Memczak, 2013). This structure confers resistance to exonucleases, giving them a longer half-life than linear RNAs (~48 hours) (Jeck, 2014).

Initially identified in viroids (Sanger, 1976), circRNAs are now recognized as regulatory elements in eukaryotic gene expression. They function as miRNA sponges, transcriptional regulators, and scaffolds for RNA-binding proteins (Ebbesen, 2016; Li et al., 2017). For instance, CDR1as harbors over 60 binding sites for miR-7, a critical regulator of neuronal development and insulin secretion (Xu et al., 2015).

circRNAs may also serve as delivery agents and play roles in antisense regulation and allosteric modulation of protein complexes (Hentze & Preiss, 2013). Many originate from protein-coding genes and consist entirely of exons (Burd, 2010; Jeck, 2014).

Biogenesis of Circular RNAs

CircRNA biogenesis involves canonical and non-canonical splicing pathways. Canonical splicing joins upstream and downstream splice sites linearly, while back-splicing connects downstream donors to upstream acceptors, forming a closed loop (Chen LL, 2015; Wang Y, 2015).

The biogenesis process is driven by intronic complementary sequences and regulated by RNA-binding proteins (Ashwal-Fluss, 2014; Conn, 2015). Models include intron-pairing-driven, lariat-driven, and RBP-driven circularization (Jeck, 2013; Wilusz, 2018).

Expression of circRNAs varies across tissues and developmental stages (Salzman, 2012; Zhang XO, 2016). Their production competes with linear splicing and is influenced by flanking intronic Alu elements, reverse complementary motifs, and RNA polymerase II activity (Zhang et al., 2014; Dong et al., 2016).

Classification and Regulatory Roles of circRNAs

Based on their origin, circRNAs are classified as:

1. **Exonic circRNAs (ecircRNAs)**: Most abundant and conserved; primarily cytoplasmic.
2. **Intronic circRNAs (ciRNAs)**: Derived from lariat introns; nuclear and regulate host gene transcription.
3. **Exon-intron circRNAs (EIciRNAs)**: Retain introns and modulate gene expression in cis and trans.

Notable examples include circEIF3J and circPAIP2, which form complexes with U1 snRNA and RNA Pol II to enhance gene transcription (Li Z, 2015; Cech, 2014). icircRNAs like ci-ankrd52 act as transcriptional enhancers by interacting with RNA Pol II (Zhang et al., 2007).

Back-splicing relies on canonical splice signals and may compete with mRNA splicing due to shared splicing factors (Starke, 2014). While ecircRNAs are cytoplasmic and may exit the nucleus during mitosis, icircRNAs remain nuclear and are resistant to debranching enzymes (Panda, 2017; Yang Zhang, 2013).

Functional Insights – Proteins and Cellular Stress

Key RNA-binding proteins such as Quaking (QKI), Muscleblind (MBL), and FUS regulate circRNA formation by binding to flanking intronic motifs (Conn, 2015; Ashwal-Fluss, 2014; Errichelli, 2017). These proteins affect epithelial-mesenchymal transitions, cardiac remodeling, and neuronal differentiation.

circRNAs modulate gene expression by competing with mRNAs for RBP binding. For instance, circFOXO3 forms a ternary complex with CDK2 and p21, halting the cell cycle (Du, 2016). circANRIL promotes apoptosis by inhibiting ribosome formation through PES1 interaction (Holdt, 2016).

Exosomal circRNA release and endonuclease degradation are two mechanisms for circRNA clearance (Lasda, 2016; Hansen, 2011). High levels of circRNAs in stressed or cancerous cells point to their regulatory role in homeostasis and tumor resistance (Bachmayr-Heyda, 2015).

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circRNAs in Disease, Aging, and Translation

circRNAs are increasingly linked to aging and disease. Expression profiles differ significantly in normal versus diseased tissues, such as tumors and lead-exposed neurons (Nan, 2016; Du, 2016b). circCDR1as regulates cell stress by sponging miR-7, which targets apoptosis-related genes like PAK1 (Fischer & Leung, 2017).

In aging, circRNA expression patterns are altered across organisms, often correlating with splicing changes (Westholm, 2014; Cortes-Lopez, 2018).

Translation of circRNAs was first observed in viroids and viruses (Kos, 1986; Perriman, 1998). Proteins like circ-ZNF609 and circMbl demonstrate cap-independent translation via internal ribosome entry sites (IRES) or m6A modifications (Abe, 2015; Legnini, 2017; Yang, 2017).

circRNAs are highly expressed in brain, liver, and heart tissues, with many neuron-specific species suggesting roles in neurodevelopment and synaptic regulation (You X, 2015; Rybak-Wolf, 2015). These findings indicate circRNAs' potential as biomarkers and therapeutic targets in metabolic, cardiac, and neurodegenerative diseases.

Reference

1. **Legnini, Ivano, et al.** “Circ-ZNF609 Is a Circular RNA that Can Be Translated and Functions in Myogenesis.” *Molecular Cell*, vol. 66, no. 1, 2017, pp. 22–37.e9. https://doi.org/10.1016/j.molcel.2017.02.017.
2. **Chen, Ling-Ling.** “The Emerging Landscape of Circular RNA in Life Processes.” *Nature Reviews Molecular Cell Biology*, vol. 17, 2016, pp. 205–211.
3. **Das, Atul, et al.** “Circular RNAs: Biogenesis, Expression and Their Potential Roles in Reproduction.” *Frontiers in Cell and Developmental Biology*, vol. 9, 2021, https://doi.org/10.3389/fcell.2021.693281.

4. **Qu, S., et al.** “Circular RNA: A New Star of Noncoding RNAs.” *RNA Biology*, vol. 14, no. 8, 2017, pp. 992–999. https://doi.org/10.1080/15476286.2016.1221004.
5. **Stoll, Livia, et al.** “Circular RNAs as Novel Regulators of ?-Cell Functions in Normal and Disease Conditions.” *Molecular Metabolism*, vol. 9, 2018, pp. 69–83. https://doi.org/10.1016/j.molmet.2018.01.009.
6. **Zhou, Chengcheng, et al.** “Identification and Characterization of Human Testis-Derived Circular RNAs and Their Existence in Seminal Plasma.” *Scientific Reports*, vol. 9, no. 1, 2019, pp. 1–12. https://doi.org/10.1038/s41598-019-46912-0.
7. **Tan, Wei, et al.** “A Landscape of Circular RNA Expression in the Human Heart.” *Cell Research*, vol. 27, no. 5, 2017, pp. 626–641.
8. **Rybak-Wolf, Anke, et al.** “Circular RNAs: Novel Regulators of Neuronal Development.” *Neuron*, vol. 90, no. 5, 2016, pp. 970–984.
9. **Zhou, Yu, et al.** “Circular RNA and Its Role in the Immune System.” *Frontiers in Immunology*, vol. 12, 2021, https://doi.org/10.3389/fimmu.2021.697183.
10. **Huang, Weizhi, et al.** “Identification of Differentially Expressed Circular RNAs in Human Monocyte-Derived Macrophages Responding to Mycobacterium Tuberculosis Infection.” *Scientific Reports*, vol. 7, 2017, https://doi.org/10.1038/srep12627.
11. **Shi, Peng, et al.** “Unique Expression Signatures of Circular RNAs in Response to DNA Tumor Virus SV40 Infection.” *Oncotarget*, vol. 8, no. 60, 2017, pp. 102987–102996. https://doi.org/10.18632/oncotarget.22156.
12. **Wang, Kai, et al.** “The Circular RNA HRCR Protects the Heart from Pathological Hypertrophy and Heart Failure by Targeting miR-223.” *European Heart Journal*, vol. 37, no. 33, 2016, pp. 2602–2611.
13. **Wang, Kai, et al.** “miR-223 Is Involved in the Pathogenesis of Cardiomyocyte Hypertrophy.” *PLoS One*, vol. 8, no. 8, 2013, e62317.
14. **Lukiw, Walter J.** “Circular RNA (circRNA) in Alzheimer’s Disease (AD).” *Frontiers in Genetics*, vol. 4, 2013, Article 307.
15. **Meng, Qing, et al.** “Circular RNAs and Their Role in Osteoarthritis and Other Lifestyle Diseases.” *Aging and Disease*, vol. 10, no. 6, 2019, pp. 1214–1223.
16. **Xia, Shuai, et al.** “Circular RNAs: Biogenesis, Function and Role in Human Diseases.” *Signal Transduction and Targeted Therapy*, vol. 4, 2019, Article 55. https://doi.org/10.1038/s41392-019-0090-5.
17. **Zhang, Xiao, et al.** “An Emerging Function of circRNA-miRNAs-mRNA Axis in Human Diseases.” *Oncotarget*, vol. 9, no. 11, 2018, pp. 10787–10798.
18. **Shen, Yuhang, et al.** “Circular RNAs and Hereditary Bone Diseases.” *Frontiers in Genetics*, vol. 10, 2019, https://doi.org/10.3389/fgene.2019.00476.
19. **Zhao, Zhenxing, et al.** “Circular RNAs and Systemic Lupus Erythematosus.” *Frontiers in Immunology*, vol. 10, 2019, https://doi.org/10.3389/fimmu.2019.00879.
20. **Hu, Xiaoping, et al.** “Circular RNAs (circRNAs) in Health and Disease.” *Clinical Epigenetics*, vol. 10, no. 48, 2018, https://doi.org/10.1186/s13148-018-0481-0.
21. **Li, Xinxia, et al.** “Circular RNAs in Cardiovascular Disease: An Overview.” *Biomedicine & Pharmacotherapy*, vol. 106, 2018, pp. 926–938.
22. **Li, Xinxia, et al.** “Biogenesis of Circular RNAs and Their Roles in Cardiovascular Diseases.” *Molecular Cell*, vol. 67, no. 2, 2017, pp. 214–227.e7.
23. **Peng, Li, et al.** “Functional Sequestration of microRNA-122 from Hepatitis C Virus by Circular RNA Sponges.” *Hepatology*, vol. 64, no. 5, 2016, pp. 1336–1347.