

Integrated Roles of RNA Biogenesis, m6A-Mediated circRNA Regulation, and Bioinformatic Analysis in Genome Integrity and Inflammation

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Abstract

Maintaining genome integrity is vital for cellular homeostasis, yet transcriptional processes inherently risk introducing mutations and recombination. A growing body of evidence highlights a critical but underappreciated interplay between RNA metabolism and DNA stability, largely mediated through the formation and resolution of R-loops—DNA–RNA hybrid structures that, when improperly regulated, can obstruct replication and repair mechanisms, leading to genomic instability. Central to this regulation are RNA biogenesis and processing factors, which act as suppressors of R-loops and modulators of transcription–replication conflicts.

Simultaneously, the post-transcriptional modification N6-methyladenosine (m6A) has emerged as a key epigenetic mark influencing RNA fate, including the biogenesis and function of circular RNAs (circRNAs). CircRNAs, characterized by their covalently closed loop structure, exhibit exceptional stability and have been implicated in gene regulation, immune responses, and disease pathogenesis. Recent findings suggest that m6A modifications not only influence the processing of linear mRNAs into circRNAs but also modulate circRNA function during inflammatory responses.

Moreover, extracellular vesicles (EVs)—including exosomes and other nanoparticle subtypes—serve as vehicles for the systemic transport of RNA species, including circRNAs and small RNAs. However, comprehensive transcriptomic profiling of EV-RNAs remains limited due to technical variability and a lack of standardized bioinformatic pipelines. Advances in high-throughput sequencing and integrative bioinformatics are crucial for decoding EV RNA cargo and harnessing its potential in diagnostics and therapeutics.

This review integrates emerging insights into RNA biogenesis, m6A-modified circRNA regulation, and extracellular RNA transcriptomics, underscoring their collective impact on genome stability and inflammation. We

also highlight the methodological challenges and provide recommendations for improving the reproducibility and accuracy of bioinformatic analyses in this evolving field.

1. RNA Biogenesis and Genome Integrity

The process of RNA biogenesis is tightly coupled with transcription and DNA replication. However, disruptions during transcription can form R-loops—structures composed of an RNA–DNA hybrid and a displaced single-stranded DNA—which, if unresolved, can threaten genome integrity by obstructing replication fork progression and repair mechanisms [1,2]. Studies have shown that components such as DDX5, RNase H1, and senataxin (SETX) play a vital role in resolving R-loops [3,4] (Table 1).

Failure of these regulators can lead to replication stress and double-strand breaks, hallmarks of genome instability in diseases such as cancer and neurodegeneration [6,7].

2. m6A Modification in circRNA Biogenesis and Function

The m6A modification is the most prevalent internal RNA modification in eukaryotic cells, significantly impacting mRNA splicing, transport, degradation, and translation [8,9]. It also influences the biogenesis of circRNAs—covalently closed, stable RNAs that arise from back-splicing events. m6A modifications promote efficient circRNA production

and alter their cellular functions, including immune response regulation and inflammation [10,11] (Table 2).

In inflammatory diseases, circRNAs such as circZNF609 and circHIPK2 have shown altered expression linked to m6A regulation, suggesting therapeutic potential [12,13]. Furthermore, m6A-modified circRNAs serve as sponges for miRNAs or protein scaffolds, modulating inflammation and immune cell signaling [14].

3. Extracellular Vesicles as RNA Carriers in Inflammation

Extracellular vesicles (EVs), including exosomes, microvesicles, and apoptotic bodies, facilitate intercellular communication by delivering nucleic acids and proteins [15,16]. EV-associated RNAs (EV-RNAs) include mRNAs, miRNAs, and circRNAs, all implicated in immune modulation and disease biomarkers. However, variability in isolation protocols and data processing has hindered reproducibility across EV studies [17].

Emerging sequencing tools and computational platforms are essential to map EV-RNA landscapes and establish their diagnostic value in inflammation and cancer [18] (Table 3).

Proper computational analysis, including normalization and quality control, is critical to interpreting the biological significance of EV-RNA cargo [19,20].

Table 1: Key RNA Biogenesis Factors Involved in R-Loop Suppression.

Factor	Function	Associated Mechanism	Reference
DDX5	RNA helicase, resolves R-loops	Transcription–replication coordination	[4]
RNase H1	Degrades RNA in RNA–DNA hybrids	Prevents R-loop accumulation	[3]
SETX	Resolves R-loops during transcription	Genome stability maintenance	[4]
TFIIS	Facilitates elongation, prevents R-loops	Suppresses transcription stress	[5]

Table 2: Key Components in m6A Modification.

Component	Role	Function	Reference
METTL3/METTL14	Writers	Catalyze m6A methylation	[9]
FTO, ALKBH5	Erasers	Demethylation of m6A marks	[9]
YTHDF1–3	Readers	Bind m6A sites, regulate RNA fate	[10]
IGF2BP1–3	Stabilizers	Enhance RNA stability via m6A	[10]

Table 3: Bioinformatic Tools for circRNA and m6A Detection.

Tool	Application	Feature	Reference
CIRI2	circRNA identification	Multiple-seed matching from RNA-seq	[21]
circExplorer2	Back-splice junctions	Annotation-based circRNA detection	[21]
exomePeak	m6A site prediction	Peak calling from MeRIP-seq data	[22]
m6Aboost	m6A site classification	Deep learning-based prediction	[22]

4. Challenges and Future Directions

Despite growing interest, significant hurdles remain in linking RNA regulatory mechanisms to disease. These include the need for:

- Standardized protocols for EV-RNA isolation
- Cross-platform reproducibility of m6A-circRNA sequencing
- Comprehensive bioinformatic pipelines for R-loop prediction

Combining molecular biology with machine learning-based analytics could yield new insights into RNA-based regulation of inflammation and genome maintenance [23,24].

5. Conclusion

The intersection of RNA biogenesis, epitranscriptomic modification, and extracellular transport provides a complex yet promising framework to understand genomic integrity and inflammatory disorders. A deeper understanding of R-loop resolution mechanisms, m6A-modified circRNAs, and extracellular RNA profiling—supported by robust bioinformatic tools—can pave the way for novel biomarkers and therapeutic strategies. Addressing current gaps in methodology and data interpretation will be key to translating these discoveries into clinical applications.

6. Conflict of Interest

The authors declare no conflict of interest.

7. References

1. García-Muse T, Aguilera A. R loops: from physiological to pathological roles. *Nat Rev Mol Cell Biol.* (2019);20(9):582–598.
2. Crossley MP, Bocek M, Cimprich KA. R-loops as cellular regulators and genomic threats. *Nat Rev Mol Cell Biol.* (2019);20(9):593–607.
3. Cerritelli SM, Crouch RJ. Ribonuclease H: the enzymes in eukaryotes. *Cell.* (2016);165(6):1233–1245.
4. Mersaoui SY, Yu Z, Coulombe Y, Karam M, Busatto FF, Masson JY. Arginine methylation of the DDX5 helicase RGG/RG motif by PRMT5 regulates resolution of RNA:DNA hybrids. *Nat Commun.* (2019);10:2711.
5. Zatreanu D, Han Z, Mitter R, Williams H, Ahmad Y, Tomkinson EM, et al. Elongation factor TFIIS prevents transcription stress and R-loop accumulation to maintain genome stability. *Nature.* (2019);571(7763):382–386.
6. De Almeida SF, García-Sacristán A, Custódio N, Carmo-Fonseca M. A link between RNA metabolism and silencing of repetitive sequences. *Nucleic Acids Res.* (2018);46(14):7075–7091.
7. Skourti-Stathaki K, Kamieniarz-Gdula K, Proudfoot NJ. R-loops induce repressive chromatin marks over mammalian gene terminators. *Nat Struct Mol Biol.* (2014);21(5):410–418.
8. Liu J, Yue Y, Han D, Wang X, Fu Y, Zhang L, et al. A METTL3-METTL14 complex mediates mammalian nuclear RNA N6-adenosine methylation. *Nat Chem Biol.* (2020);16(2):129–136.
9. Yang Y, Fan X, Mao M, Song X, Wu P, Zhang Y, et al. Extensive translation of circular RNAs driven by N6-methyladenosine. *Mol Cell.* (2018);71(3):428–442.e5.
10. Zaccara S, Ries RJ, Jaffrey SR. Reading, writing and erasing mRNA methylation. *Nat Rev Mol Cell Biol.* (2019);20(10):608–624.
11. Chen RX, Chen X, Xia LP, Zhang JX, Pan ZZ, Ma XJ, et al. N6-methyladenosine modification of circNSUN2 facilitates cytoplasmic export and stabilizes HMGA2 to promote colorectal liver metastasis. *Mol Cancer.* (2020);19:126.
12. Zhang X, Yang H, Zhao J, Fan D. m6A regulator-mediated RNA methylation modulates human macrophage inflammatory response through the circZNF609-miR-145-MYD88 axis. *Front Immunol.* (2021);12:672802.
13. Zhou C, Molinie B, Daneshvar K, Pondick JV, Wang J, Van Wittenberghe N, et al. Genome-wide maps of m6A circRNAs identify widespread and cell-type-specific methylation patterns that are linked to function. *Cell Death Dis.* (2021);12(6):573.
14. Kristensen LS, Andersen MS, Stagsted LVW, Ebbesen KK, Hansen TB, Kjems J. The biogenesis, biology and characterization of circular RNAs. *Nat Rev Genet.* (2019);20(11):675–691.
15. Kalluri R, LeBleu VS. The biology, function, and biomedical applications of exosomes. *Science.* (2020);367(6478):eaau6977.
16. Théry C, Witwer KW, Aikawa E, Alcaraz MJ, Anderson JD, Andriantsitohaina R, et al. Minimal information for studies of extracellular vesicles 2018 (MISEV2018): a position statement. *J Extracell Vesicles.* (2018);7(1):1535750.
17. Van Deun J, Mestdagh P, Agostinis P, Anckaert J, Martinez Z, Baggherman G, et al. EV-TRACK: transparent reporting and centralizing knowledge in extracellular vesicle research. *Nat Methods.* (2017);14(3):228–232.
18. Yáñez-Mó M, Siljander PR, Andreu Z, Zavec AB, Borràs FE, Buzas EI, et al. Biological properties of extracellular vesicles and their physiological functions. *J Extracell Vesicles.* (2015);4:27066.
19. Li S, Li Y, Chen B, Zhao J, Yu S, Tang Y, et al. exoRBase: a database of circRNA, lncRNA and mRNA in human blood exosomes. *Nat Commun.* (2019);10:2484.

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20. O'Brien K, Breyne K, Ughetto S, Laurent LC, Breakefield XO. RNA delivery by extracellular vesicles in mammalian cells and its applications. *J Extracell Vesicles*. (2020);9(1):1706709.
 21. Zhang XO, Dong R, Zhang Y, Zhang JL, Luo Z, Zhang J, et al. Diverse alternative back-splicing and alternative splicing landscape of circular RNAs. *Cell*. (2016);166(1):289–302.
 22. Liu S, Zhu A, He C, Chen M, Gao J, Xu Y, et al. m6Aboost: a deep learning-based framework for identifying and analyzing m6A sites from MeRIP-seq data. *Nat Commun*. (2021);12:3257.
 23. Parker MT, Knop K, Sherwood AV, Schurch NJ, Mackinnon K, Gould PD, et al. Nanopore direct RNA sequencing maps the complexity of Arabidopsis mRNA processing and m6A modification. *Genome Biol*. (2020);21(1):269.
 24. Zeng Y, Wang S, Gao S, Gao H, Qian J, Zhang W, et al. Comprehensive bioinformatics analysis and experimental validation of potential circRNA–miRNA–mRNA networks in inflammatory diseases. *Front Immunol*. (2022);13:826172.