



Views & Comments

Can DNA Be Glycosylated?

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The central dogma of molecular biology—detailing the unidirectional flow of genetic information from DNA to RNA to proteins—has long been regarded as the definitive framework for understanding biological processes [1]. While this concept has proven invaluable, it leaves many cellular phenomena unexplained. Recent discoveries in glycobiology challenge this framework by introducing the paracentral dogma, which positions glycans as the third alphabet of life, complementing nucleic acids and proteins (Fig. 1) [2,3]. The finding that RNA molecules can be glycosylated has redefined our understanding of molecular interactions [4,5]. But what about DNA? Could it, too, undergo glycosylation? This question lies at the intersection of emerging research in glycomics, glycomedicine, and fundamental inquiries into cellular biology.

This article explores the potential of DNA glycosylation, beginning with the established role of glycosylation in RNA, examining the mechanisms and biological significance of these modifications, and considering whether similar processes might apply to DNA. By juxtaposing the central dogma with the paracentral dogma [1,2], we illuminate new dimensions of molecular biology, offering a roadmap for future research into this uncharted territory.

2. RNA glycosylation: A new molecular frontier

RNA glycosylation represents a paradigm-shifting discovery in molecular biology, significantly expanding our understanding of RNA functionality. Small noncoding RNAs, including small nuclear RNAs (snRNAs), ribosomal RNAs (rRNAs), small nucleolar RNAs (snoRNAs), transfer RNAs (tRNAs), Y-RNAs, and microRNAs, have been found to undergo *N*-glycan modifications, such as sialylation and fucosylation. These glycosylated RNAs exhibit distinct molecular functions compared to their non-glycosylated counterparts [4,5]. GlycoRNAs—RNAs covalently modified with *N*-glycans—have challenged the conventional view that glycosylation is restricted to proteins and lipids, revealing RNA as a dynamic substrate for glycosylation [4]. This discovery highlights the intricate interplay

between glycobiology and RNA biology, opening a new avenue for research in molecular communication and cellular regulation.

2.1. Mechanism of RNA glycosylation

RNA glycosylation involves the attachment of glycan moieties to specific RNA bases, fundamentally altering their chemical properties and biological functions. The study by Xie et al. [6] identified 3-(3-amino-3-carboxypropyl) uridine (acp3U) in tRNAs as a conserved site for *N*-glycan attachment. This modification depends on canonical glycosylation machinery, including enzymes such as glycosyltransferases, which traditionally mediate protein glycosylation.

The specificity of this process suggests that RNA glycosylation is tightly regulated and may involve additional factors such as chaperones, cofactors, or RNA-binding proteins that ensure precise modification. Moreover, the existence of conserved glycosylation sites like acp3U across various cell types and organisms

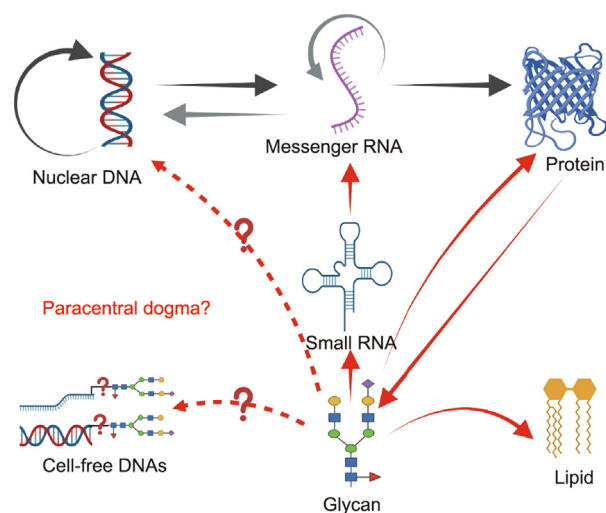


Fig. 1. Central dogma and paracentral dogma concepts in molecular biology. While the central dogma of molecular biology outlines the linear flow of genetic information from DNA to RNA to proteins (black lines), glycomics introduces a “3rd code of life”—glycans—that operates outside the bounds of pre-defined genetic templates (red lines).

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underscores its evolutionary importance [4,6]. Notably, such sugar modifications have been detected in various mammalian cell lines, including human embryonic kidney cells extensively utilized for protein expression and genetic transfection (HEK293T cells), human embryonic stem cells characterized by specific spatial and temporal expression patterns (H9 ES cells), and an immortalized cervical cancer cell line frequently employed in oncological studies (HeLa cells) [4]. These findings challenge traditional perspectives, inviting further investigation into how the glycosylation machinery identifies and modifies RNA substrates.

Detecting glycoRNAs required innovative methodologies due to their low abundance and the technical challenges associated with studying glycans attached to RNA. Techniques like RNA-optimized periodate oxidation and aldehyde labeling (rPAL) have enabled selective enrichment and labeling of glycoRNAs. When coupled with sequential window acquisition of all theoretical mass spectra (SWATH-MS), researchers achieved unprecedented sensitivity and specificity in identifying glycoRNAs [4,6]. This methodological advancement has revealed the widespread presence and diversity of glycoRNAs, highlighting their biological relevance.

2.2. Biological significance

GlycoRNAs are predominantly localized on the surface of living cells, where they serve as molecular interfaces for interactions with other biomolecules, particularly immune receptors such as sialic acid-binding immunoglobulin-like lectins (Siglecs). These receptors play critical roles in recognizing glycans and mediating immune responses. The engagement of Siglecs with glycoRNAs modulates inflammation, pathogen recognition, and immune tolerance, underscoring glycoRNAs' functional significance [4].

The dual composition of glycoRNAs—combining RNA sequences with complex glycan structures—enables them to mediate diverse biological functions. The RNA component allows sequence-specific interactions with other RNAs, proteins, or ribonucleoprotein complexes, while the glycan moiety facilitates structural recognition by glycan-binding proteins. This dual functionality positions glycoRNAs as versatile molecules capable of integrating genetic information with cellular signaling networks [4,6].

Importantly, glycoRNAs may act as biomarkers for cellular health and disease. Aberrant glycosylation patterns on RNA could signal pathological states, such as cancer or autoimmune disorders [4,6]. For example, specific glycoRNA profiles may reflect alterations in glycosylation machinery due to genetic mutations or environmental stressors. Thus, glycoRNAs hold promise for use in diagnostic applications and as therapeutic targets.

Beyond immune regulation, glycoRNAs likely participate in intercellular communication. Their surface localization suggests a role in transmitting signals between cells, potentially influencing processes like tissue development, wound healing, or tumor progression. Furthermore, pathogens that mimic glycoRNA structures may exploit these interactions to evade immune detection, revealing a possible co-evolutionary relationship between host and pathogen [4,6].

The discovery of glycoRNAs also highlights their potential in therapeutic interventions. Modulating glycoRNA pathways—through glycosylation inhibitors, engineered glycoRNAs, or Siglec-targeting therapies—could provide novel treatments for immune-related diseases and cancers. For example, selectively altering glycoRNA interactions with Siglecs might suppress inflammation or enhance immune clearance of pathogens [4,6].

Finally, glycoRNAs prompt a reevaluation of RNA's roles in cellular biology. Traditionally regarded as intermediaries in genetic information flow or as functional ribozymes, RNAs now emerge as key players in glycan-mediated processes. This functional versa-

tility underscores the need to integrate RNA biology with glycobiology, fostering interdisciplinary approaches to unravel the complexities of molecular life.

In summary, RNA glycosylation represents a transformative addition to our understanding of cellular biology. By bridging the fields of glycobiology and RNA research, glycoRNAs offer new insights into immune regulation, disease mechanisms, and potential therapeutic strategies, cementing their place as critical molecular players in the paracentral dogma.

3. Expanding the horizon: DNA and glycosylation

While DNA glycosylation has not yet been experimentally confirmed, it presents an intriguing area for research. Traditionally, glycosylation is associated with proteins, lipids, and recently RNA, but it may also extend to DNA under specific biochemical or cellular conditions (Fig. 2). If proven, DNA glycosylation could introduce new functional and structural roles within the cell.

3.1. Distinguishing DNA glycosylation from DNA in glycosylation

Before exploring the potential mechanisms and functions of DNA glycosylation, it is important to distinguish it from DNA's role in glycosylation, similar to the differentiation between glycoRNA and RNA in glycosylation [7].

DNA glycosylation, if it exists, would involve the direct enzymatic addition of glycans to DNA, akin to RNA glycosylation. This modification could impact DNA structure, gene regulation, and cellular functions. Although no direct evidence currently supports enzymatic DNA glycosylation, non-enzymatic DNA glycation demonstrates that sugar modifications on DNA are chemically feasible, suggesting the possibility of enzymatic mechanisms yet to be discovered. Exploring DNA glycosylation in various forms of DNA—such as nuclear DNA, mitochondrial DNA, plasmid DNA, and cell-free DNA—offers critical insights into cellular processes and potential therapeutic targets [8].

In contrast, DNA in glycosylation refers to the role of DNA in encoding and regulating glycosylation-related processes. This includes the genetic regulation of enzymes essential for glycan biosynthesis, such as glycosyltransferases, glycosidases, and nucleotide sugar transporters, which are responsible for protein, lipid, and RNA glycosylation. Mutations in glycosylation-related genes can lead to congenital disorders of glycosylation (CDGs) and other metabolic diseases. Genomic sequences of various organisms contain glyco genes that encode a diverse array of enzymes involved in sugar metabolism. To date, more than 180

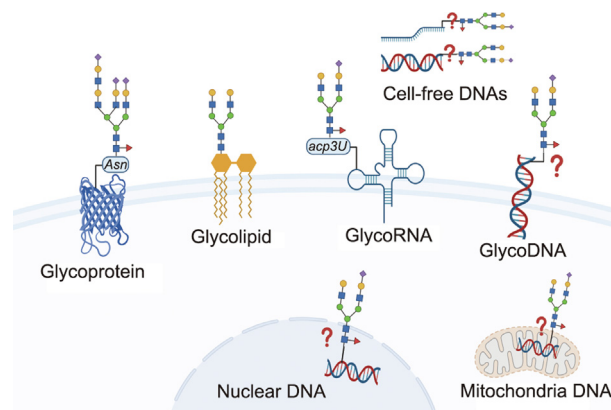


Fig. 2. Diverse structures of glycoconjugates with protein, lipid, small RNA, and DNA. Glycans modify proteins, lipids and small RNAs, playing an essential role in cellular functions and signaling. Nuclear DNAs, mitochondria DNA, and cell-free DNAs may serve as significant targets for glycosylation. Asn: asparagine.

glycogenes linked to human biological processes have been identified, cloned, and extensively characterized (GlycoGene DataBase).

3.2. Structural and chemical challenges

DNA is a chemically stable molecule, characterized by the deoxyribose sugar in its backbone [9]. Unlike RNA, DNA lacks the reactive hydroxyl group at the 2' position of its sugar moiety, which is a critical site for many biochemical modifications. This absence makes DNA less amenable to typical glycosylation reactions. Furthermore, the double-helical structure of DNA, with its tightly packed base pairs, limits accessibility to potential modification sites [9].

However, DNA's phosphate backbone and exposed nitrogen atoms in its bases could, under the right enzymatic or environmental conditions, serve as attachment points for glycans. These unique structural features might facilitate non-traditional glycosylation mechanisms, potentially mediated by novel enzymes or under specific cellular contexts, such as stress responses, chromatin remodeling, or during DNA repair processes [1,9].

3.3. Evidence from non-enzymatic glycation

While enzymatic DNA glycosylation remains hypothetical, non-enzymatic glycation provides indirect evidence of sugar–DNA interactions. DNA glycation is a spontaneous non-enzymatic amino-carbonyl reaction that occurs when reducing sugars react with DNA, leading to the formation of advanced glycation end products (AGEs).

In pathological conditions like diabetes, elevated levels of glucose and its reactive derivatives lead to DNA glycation, which has been linked to increased mutation rates, compromised DNA stability, and accelerated aging-related processes [10]. These modifications can result in: ① cross-linking of DNA strands, DNA–protein interactions, and disruption of chromatin structure and normal cellular function; ② accumulation of glycated DNA adducts, which have been implicated in cancer, cardiovascular diseases, and neurodegenerative disorders.

While glycation differs mechanistically from enzymatic glycosylation, its existence underscores the susceptibility of DNA to sugar-related chemical alterations and raises the question of whether enzymatic pathways might similarly target DNA for glycosylation.

3.4. Hypothetical mechanisms of DNA glycosylation

If DNA glycosylation occurs, it would likely involve specialized enzymatic machinery adapted to DNA's unique structure. Glycosyltransferases, the enzymes responsible for transferring sugar moieties to acceptor molecules, are typically associated with proteins and RNA. A hypothetical DNA-specific glycosyltransferase would need to: ① recognize DNA as a substrate amidst the molecular complexity of the cell; ② identify specific sites on DNA bases or the phosphate backbone for glycan attachment; and ③ operate within the constraints of chromatin organization, which limits enzyme access to DNA.

Such enzymes might function in specific cellular compartments, such as the nucleus or mitochondria, and could be regulated by factors such as cell cycle phase, DNA damage, or metabolic state. Advances in structural biology and enzymology could uncover whether glycosyltransferases with these capabilities exist.

3.5. Potential cellular roles

If DNA glycosylation were proven, it could revolutionize our understanding of DNA's role beyond genetic information storage.

Possible functions include: ① Epigenetic regulation and cellular information storage: Glycosylated DNA could serve as a novel layer of epigenetic modification, influencing chromatin structure and gene expression. “The Cellular Dogma” suggests genetic information alone may not be sufficient to define cell identity, implying the existence of additional regulatory mechanisms [11]. Glycosylated DNA might interact with histones, transcription factors, or chromatin remodeling complexes, modulating accessibility to transcriptional machinery. Furthermore, if glycosylation marks are inherited across cell divisions, they could provide an additional form of cellular memory, contributing to long-term epigenetic inheritance and cellular identity regulation. ② DNA repair and stability: Glycosylation might play a protective role, shielding DNA from oxidative damage or facilitating repair processes. ③ Immune recognition: Similar to glycoRNAs, glycosylated DNA could participate in immune surveillance, distinguishing self from non-self during immune responses or pathogen recognition. ④ Cell–cell communication: Surface-localized glycosylated DNA, if present, could mediate intercellular communication, similar to the roles observed for glycoRNAs.

3.6. Experimental evidence and future directions

Although the evidence for DNA glycosylation is currently circumstantial, advances in glycomics and molecular biology are likely to shed light on this possibility. Tools such as glycan-specific antibodies, glycosylation inhibitors, microarray, high pressure liquid chromatography, high-resolution mass spectrometry, and cryo-electron microscope could be employed to detect, image, and characterize potential DNA glycosylation events. Furthermore, genome-editing technologies like clustered regularly interspaced short palindromic repeats (CRISPR)–CRISPR-associated protein 9 (Cas9) could be used to manipulate genes encoding glycosylation enzymes, probing their effects on DNA and cellular functions.

The discovery of glycoRNAs has demonstrated that seemingly rigid biological paradigms can be overturned. By investigating the possibility of DNA glycosylation, researchers may uncover novel pathways and molecular interactions that expand our understanding of DNA's role in cellular biology and disease. Whether glycosylation will join methylation, acetylation, and phosphorylation as a recognized DNA modification remains an open and exciting question for future research.

In summary, while DNA glycosylation is currently speculative, its exploration could reveal transformative insights into the molecular mechanisms governing life, bridging the gap between the central and paracentral dogmas of molecular biology [1–3].

4. Conclusion

The question of whether DNA can be glycosylated remains unanswered but is deeply compelling. The discovery of RNA glycosylation has already transformed our understanding of molecular biology, highlighting the dynamic interplay between nucleic acids and glycans. Extending this research to DNA could uncover new dimensions of cellular communication and molecular regulation, bridging the central and paracentral dogmas.

As glycomedicine continues to expand, the exploration of DNA glycosylation represents a frontier ripe for discovery. By embracing the complexity and interconnectedness of life's molecular building blocks, we can chart a path toward a more holistic understanding of biology and unlock new possibilities for therapeutic innovation.

In the words of those who pioneered this field, life's sweet secrets are waiting to be uncovered—perhaps even within the DNA double helix itself [12].

Acknowledgments

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