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The Much-Awaited Bridge: Connecting the Worlds of RNA Biology and Glycobiology through the Discovery of GlycoRNAs

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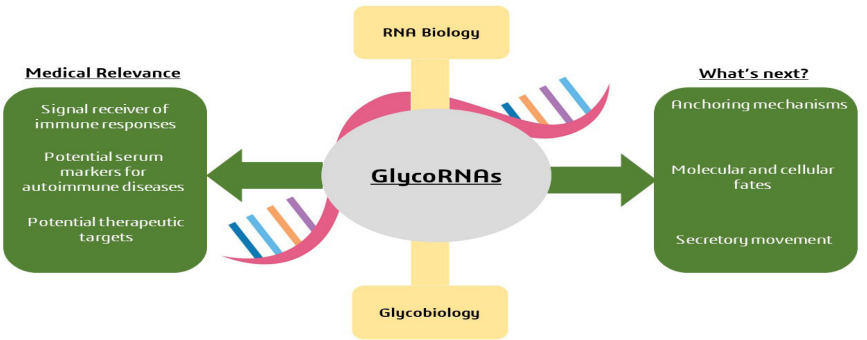
Graphical Abstract



Abstract

Glycosylation is a molecular process known to occur in lipids and proteins. Until recently, a new class of non-coding RNAs called glycoRNAs was discovered using bioorthogonal chemistry approaches. In this review paper, fundamental concepts in traditional RNA biology and glycobiology are reviewed. From the conventional way of studying RNA biology which is centered on its functions, structures, regulation, and synthesis, recent studies on RNA are now shifting to epigenetics and omics, especially its influence on disease pathogenesis and the process itself of post-transcriptional and post-translational modifications. In the study of glycobiology, recent investigations are centered on the pathophysiological relevance of the process of glycosylation. The discovery of glycoRNAs moves forward research on both RNA biology and glycobiology starting again from basic science. It establishes the foundation of future scientific endeavors which aims to clarify and answer unclear concepts such as glycoRNA trafficking within cells and the fate of glycoRNAs in the extracellular space. We also highlighted in this paper the promising potentials of glycoRNAs in both molecular diagnostics and therapeutics against autoimmune diseases.

Keywords: glycans; ribonucleic acids; glycosylation; Siglec receptors; glycoRNAs

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Introduction

Glycosylation is a well-known process in cell biology where sugar moieties covalently bind to a polypeptide chain thereby inducing post-translational modification. This process is both established to occur in lipids and proteins. Until recently, ribonucleic acids (RNAs) have been discovered to functionalize with sugar moieties thus coining the term "glycoRNAs" [1]. In this paper, the once separate yet adjacent worlds of RNA biology and glycobiology are bridged through comprehensive discussion of this breakthrough. To synthesize the impact of the discovery of glycoRNAs to the field of cell and molecular biology, fundamental concepts in RNA biology and the process of glycosylation are reviewed followed by an extensive discussion of the paper highlighting the newly discovered glycoRNAs. This review paper encompasses concepts in RNA biology that are deemed necessary to grasp the importance of the discovery of glycoRNAs.

Molecular Biology of RNA. Ribonucleic acid (RNA) is a type of nucleic acid which utilizes deoxyribonucleic acid (DNA) template for protein synthesis. While it is well-established in the study of the central dogma of life that DNA is responsible for carrying genetic information, DNA itself still requires transcription into RNA to enable gene expression [2,3]. Thus, it is thought that RNA is more ancient that DNA. According to the RNA world hypothesis, RNA is the first "genetic blueprint" of life on Earth which descends from ancient microbes capable of RNA-dependent replication known as ribocytes [4]. RNA still works independently as a genome in the present time as observed in some viruses like human immunodeficiency virus (HIV) and influenza [5].

Due to the reliance of RNA to its parent DNA template for its synthesis, there is an overlapping set of bases for both nucleic acids. However, the clear difference between the two is that RNA uses uracil as one of its four bases instead of thymine in DNA. Thymine provides DNA greater chemical and structural stability than uracil and is used by DNA in repair of genetic damage. Despite the more unstable nature of RNA, it has a variety of functions that maintain the normal physiology and biochemistry of cells (Table 1) [6,7].

RNAs can be classified into three main types depending on its function in protein synthesis: messenger RNA (mRNA), transfer RNA (tRNA), and ribosomal RNA (rRNA) [16] RNAs can also be divided into much broader classifications depending on its coding capability: the coding RNA (cRNA) and noncoding RNA (ncRNA). cRNAs are composed of mRNAs [7,17] (Figure 1). Meanwhile up to 85% of the human genome is transcribed into RNA and is known as the transcriptome [18]. However, a large proportion of the transcriptome is composed of ncRNAs. In humans, there is a 47:11 ratio of ncRNA to cRNA. Meanwhile, the ratio for mice is 43:1 and 2.4:1 for *Drosophila melanogaster* [19,20]. Despite this difference in proportion, the abundance of ncRNAs is well-established across species and this prompted several RNA biologists to investigate them and their role in regulating gene expression [21-24].

Table 1. Functions of RNAs and some examples in cellular and physiological processes.

Functions of RNA	Examples of cellular and physiological processes	References
RNAs are carriers of genetic information instead of DNAs.	Entirely RNA genomes of some viral classes RNA as genetic material in evolutionary studies mRNAs in the central dogma	[8]
RNAs can have recognition functions through base pairing.	splice site recognition snoRNAs and substrate rRNAs miRNAs and corresponding mRNAs	[9,10]
RNAs have catalytic properties.	Ribozymes	[11]
Specific shapes correspond to docking functions.	RNA-induced interactions between ribosomal subunits and between RNA aptamers and ligands	[12,13]
Large ribonucleoprotein complexes are built through scaffolding RNAs.	Spliceosome Signal recognition particles and ribosomes	[3,14]
RNAs can be templates for nucleotide synthesis.	Telomerase RNAs as templates for DNA synthesis	[15]

The ability of the RNA to fold into diverse structural entities gives it several functions in the cell. There are three hierarchical levels of RNA structural organization. Figure 2 provides a systematic workflow of how RNA molecules form structures starting from the primary to the tertiary structure in comparison with DNA structures. The key difference between DNA and RNA is observed in the secondary structure. Tertiary structure does not occur in DNA molecules [6,25-27]. Due to the ability of RNAs to fold into tertiary structures, RNA can interact with other biological molecules such as other RNA molecules, DNA and proteins. These RNA-associated interactions are involved in several physiological and cellular processes like cell growth, cell differentiation, and apoptosis [28]. For example, mRNA-miRNA interactions yield mRNA degradation while tRNA-mRNA interaction results in amino acid synthesis [29-31]. RNAs can also provide catalytic activities in ribozymes. The ability of RNA to bind metal ions, its unique bases which can accept and donate protons, and the presence of 2'-OH groups on RNA ribose also contribute to its enzymatic properties [32-34].

In the era of transcriptomics, studies have been focused on RNA editing or RNA modifications with over 140 types of RNA modification [35]. The usual 5' cap and 3' poly(A) tail modification of mRNA has been extensively investigated and sparked research on post-transcriptional modifications. Due to the role of these modifications in RNA functioning, structural stabilities, and correct biogenesis, other primary classes of RNA modifications have been identified in the last 20 years which include adenosine methylation, cytosine modifications and editing, ribose modifications, adenosine methylation, and cytidine acetylation [36-39]. Through studies on post-transcriptional modifications, the role of RNA in the regulation of protein synthesis and gene expression via splicing, translation, and decay has been clearer and more accepted in the scientific community [40]. In addition to this wide array of chemical modifications of the ribose group and RNA nucleosides, the team of Flynn and colleagues [1] discovered glycosylation in RNA molecules which expands current RNA epigenetic studies.

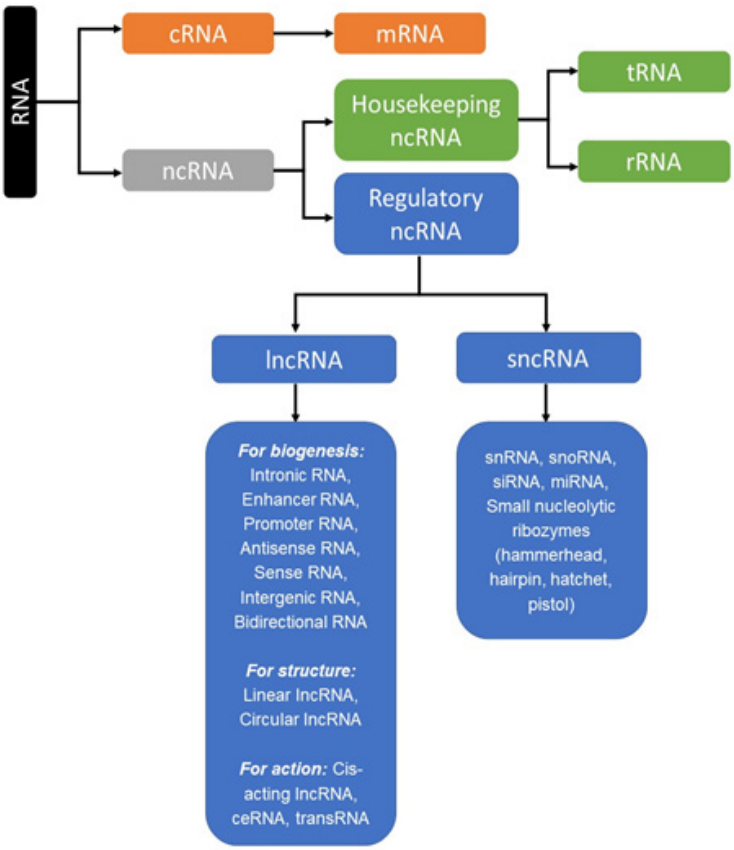

Figure 1. Classification and systematics of RNA. Abbreviations: Coding RNA (cRNA); Non-coding RNA (ncRNA); Transfer RNA (tRNA); Ribosomal RNA (rRNA); long ncRNA (lncRNA); small ncRNA (sncRNA); Competing endogenous RNA (ceRNA); trans-acting RNA (transRNA); small nuclear RNA (snRNA); small nucleolar RNA (snoRNA); small-interfering RNA (siRNA); microRNA (miRNA).

Glycans and the Process of Glycosylation. Glycans or carbohydrate-based polymers (polysaccharides) are produced by all living organisms. These are biomolecules essential in structure, energy storage and regulatory purposes at the cellular and systems level [41]. Glycans are arguably the most abundant and diverse polymers among the four basic constituents of cells [42]. Seven monosaccharides assemble to synthesize glycans: fucose, sialic acid, glucose, mannose, galactose, *N*-acetylglucosamine, and *N*-acetylgalactosamine. Fucose and sialic acid occur at the terminal ends of glycan chains and attach to hydroxyl groups present in the protein [43].

The glycome, the entirety of sugar moieties in an organism, is so diverse with different types of oligosaccharides and glycoconjugates with a myriad of linkages and sequences involved in the binding. Amidst this chemical and biochemical diversity among glycans, glycoconjugates have some similarities in their terminal modifications and structural scaffolds [44]. The differences meanwhile are thought to be the result of eukaryotic evolution due to molecular cues and the need for regulatory processes [45-46].

The covalent attachment of these glycans to other macromolecules like proteins and lipids is called glycosylation. In proteins, glycosylation is the most abundant and second most studied post-translational modification after phosphorylation [47]. Most eukaryotic proteins synthesized in the ribosomes undergo this process via *N*-linked glycosylation to asparagine residues or *O*-linked glycosylation to serine and threonine. Protein glycosylation is a complex cellular process that undergoes several biochemical steps either through non-enzymatic reactions (glycation) or with enzymes. The earlier involves a reaction between aldehyde glucose with amino acid residues lysine and arginine to stimulate the production of advanced glycation end products which are implicated in several pathological and physiological processes like aging, cancer, and [47-48]. The enzymatic protein glycosylation or simply glycosylation requires sequential steps in the secretory pathway (endoplasmic reticulum and Golgi bodies), nucleus, cytoplasm, and mitochondria. The process employs an estimate of 200 glycosyltransferases [49-50]. Glycosylation amplifies the proteome through synthesis of several proteoforms with diverse properties which equate to a wide array of functions [51-53]. Despite this diversity, glycosylation pathways are still considered similar among mammalian systems except for some eliminated glycan features through gene inactivation and as observed in xenoantigens [54-56].

Through thermodynamics analysis, glycosylation has been found to stabilize proteins by reducing the integrity of unfolded proteins [57]. For example, *N*-glycosylation allows correct protein folding into its three-dimensional form thereby promoting biological functions like cell signaling and cell-cell communication [58-59]. Glycosylated proteins are also more stable and have longer half-lives after *N*-linked glycosylation which prevents glycoprotein deamidation [60-62]. Furthermore, protein glycosylation also influences protein degradation and trafficking [63].

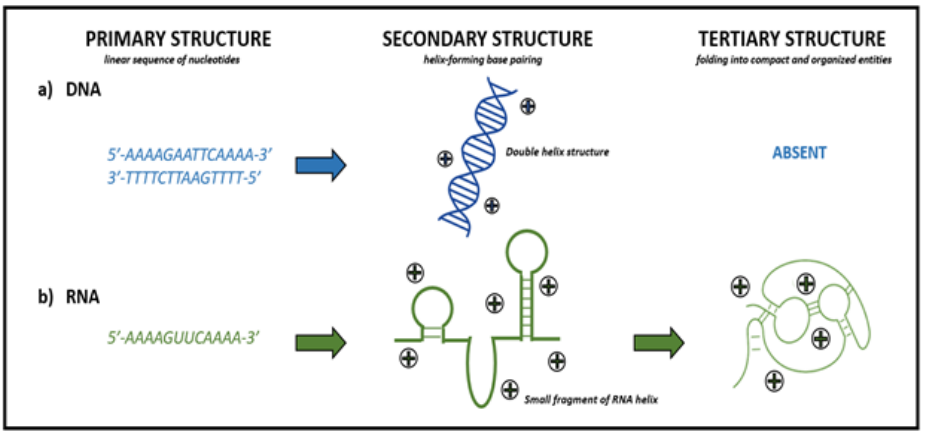

Figure 2. Systematic workflow of the three levels of structural organization in (a) DNA and (b) RNA. Both primary structures of DNA and RNA are linearly arranged sequences of nucleotides. DNA secondary structure features a stable double helix with the aid of positively charged (+) molecules. Secondary structures of RNA are due to double-stranded RNA helices. Only RNA forms a tertiary structure after folding and packing the helices.

Glycosylation can influence ligand-receptor interactions which consequently affect signal transduction. Thus, glycosylation is implicated in pathophysiology of several diseases. Sialylation (sialic acid-binding) promotes the development of galectin-1 dependent anoikis resistance among tumor cells by combining with and eventually switching off the signal of a glycan receptor [64]. Sialylation and fucosylation (fucose-binding) of glycosylation-dependent cell adhesion entities promote lymphocyte homing [65]. Glycans can also be antigenic where blood-group antigens are produced by residues bound to sialic acid and fucose [66-67].

Glycosylation is also associated with multidrug resistance. Acute myeloid leukemia cells may develop chemotherapy resistance after binding to endothelial E-selectin receptors via glycosylation induced by sialyl-transferase and [68-69]. *N*-glycosylation modifications may promote cancer malignancy through the CD63 protein which recruits receptor tyrosine kinases to integrins and kinases belonging to the Src family [70]. Glycosylated CD63 proteins are known to possess drug resistance [71].

Discovery of GlycoRNAs. As stipulated in the discussions above, glycosylation typically occurs on proteins, lipids and glycans itself [72-73]. RNA-targeted glycosylation is uncommon although there have been studies investigating glycosylation-associated ncRNA. In the study of Flynn et al. [1], RNAs are directly incorporated into the world of glycans and thus they coined the term "glycoRNAs". GlycoRNAs are members of ncRNAs but with a distinct overrepresentation of Y RNAs and small nucleolar RNA (snoRNA). Y RNAs are constituents of Ro6 ribosomal nucleoproteins while snoRNAs are well-documented in post-transcriptional modifications [74-75]. RNA glycosylation was investigated using biorthogonal chemistry techniques developed by the Bertozzi laboratory [76]. Azido-sugars were utilized to visualize and trace sugar moieties and glycoconjugates [77]. In the study, it was found that PNGase F, an enzyme which catalyzes the cleavage of linkage between asparagine residues and the *N*-linked glycans specifically the proximal *N*-acetylglucosamine, can digest glycoRNAs. Thus, RNA glycosylation uses an amide bond for its linkages. The authors confirmed that it is RNA that interacted with cellular glycans through biotin reactivity tests of labeled cells, which was reversed by treatment with RNAse. Furthermore, these glycoRNAs are highly fucosylated and sialylated. However, RNA nucleobases do not have amide linkers thereby suggesting potential modifications among precursors of these nucleobases to resemble asparagine structure and function in glycosylation.

Evidence suggests that glycoRNA glycans are similar to protein glycans in terms of their structures. Despite unclear biosynthetic pathways of glycoRNA, enzymes which function in its synthesis are comparable and may be similar to enzymes involved in the synthesis of *N*-glycans such as transferases. Transferases, which are key enzymes participating in protein glycosylation, may shed light to these similarities between RNA and glycans found in glyco-RNAs. Only *N*-glycosylation enzymes can control glycoRNA biosynthesis and *N*-glycans are the only glycan structures on glycoRNAs. These enzymes are localized in both ER and Golgi bodies [78-80]. Meanwhile, small ncRNAs occur at the cytosol and its modification-inducing enzymes are in the lumen of ER and Golgi bodies. This suggests potential translocation of small ncRNAs to the location of these enzymes to synthesize glycoRNAs.

The study also found that these glycoRNAs localize to the cell membrane. To reach the cell surface, glycoRNAs rely on the secretory pathway. With glycoRNAs at the cell surface, their roles in cell-to-cell communication and interactions by acting as ligands to target receptors on other cells and chemical moieties might be pathophysiologically important. The recent discovery of Flynn and colleagues proposed that glycoRNAs may serve as ligands for Siglec receptors therefore glycoRNAs might have significant functions in immune signal transduction. Siglec receptors are immune receptors for autoantibodies thus highlighting its medical importance especially in autoimmune diseases like systemic lupus erythematosus [81].

Prior to discovery of glycoRNAs, the relationship between glycans and RNA has long been investigated. However, it is the first time to confirm that glycans bind to RNAs. For the past decades, glycosylation-associated ncRNAs have been the focus of scientists who try to bridge RNA biology and glycobiology as well as elucidate their medical relevance. Glycosylation-associated ncRNAs and glycoRNAs are two different fields and studies on the earlier paved the way for the discovery of the latter. These glycosylation-associated ncRNAs function to influence and alter protein glycosylation patterns, regulate the enzymatic activity of glycosyltransferase, and control glycan-associated protein expression. For instance, lncRNA SNHG7 has been found to be a competing endogenous RNA that cleans RNAs from the microRNA (miRNA) 34a family thereby inhibiting the binding of *N*-acetylgalactosamine to these miRNAs. This phenomenon leads to cancer proliferation and metastasis [82]. These ncRNA-associated glycans are not directly bound to ncRNAs. The first report of ncRNA-glycan linkage yields a new and distinct class of ncRNA – the glycoRNAs.

Future Directions of the Discovery . In terms of the medical relevance of glycoRNAs, the role of glycoRNAs in immunotherapy is now becoming the emphasis of current studies. GlycoRNAs may serve as serum markers for autoimmune diseases. RNA modifications through glycosylation are reported to be sensitive to immunotherapeutic armamentarium thus glycoRNAs may also be good therapeutic targets. Alteration of glycan structures in glycoRNAs may influence epigenetic mechanisms involved in the development of lesions in systemic lupus erythematosus [83]. GlycoRNAs may be the next signal receiver of immune responses to chemotherapeutic drugs in addition to protein receptors.

Other than the potential medical importance of glycoRNAs, the study also provided a new set of research concepts for future studies. The process of translocation of ncRNAs into the ER lumen and the movement of the glycoRNAs via secretory pathway remain unclear. Cargo complexes involved in glycoRNA trafficking from RNA synthesis in the nucleus to its end destination, which is the cell surface, need to be elucidated. The mechanism of anchoring of glycoRNAs at the cell surface and its high specificity to this location may also be investigated. Lastly, future studies on the fate of these glycoRNAs at the cell surface, whether they are endocytosed or simply released and dissolved extracellularly, might strengthen its medical relevance.

Conclusion

The recent discovery of glycoRNAs paved the way for the establishment of a new class of ncRNAs. GlycoRNA, which is formed via direct linkage between *N*-glycans and ncRNAs, is the answer to the missing bridge between RNA biology and glycobiology. The difference between glycosylation-associated RNA and glycoRNA is that the earlier are RNAs involved in the process of glycosylation while the latter is the RNA itself being glycosylated. Both have medical importance but glycoRNAs might potentially be both a molecular diagnostic marker and therapeutic target for autoantibody diseases in the future. Despite the limitation of glycoRNAs in mammalian cells, the discovery may allow scientists to use glycoRNA data in studying traditional RNA biology, the process of glycosylation and glycobiology, as well as the recent trends in glycomics, and other omics workflows in cell and molecular biology.

Acknowledgment

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Conflict of Interest

The authors declare no conflict of interest.

Author Contributions

Conceptualization, J.A.H.M; Gathering of related literature, J.A.H.M. and M.B.B.M; Analysis and synthesis, J.A.H.M. and M.B.B.M; Original draft preparation, J.A.H.M. and M.B.B.M; Review and editing of the draft, J.A.H.M. and M.B.B.M. Both authors have read and agreed to the final version of the manuscript.

Institutional Review Board Statement

Not applicable.

Informed Consent Statement

Not applicable.

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