Exploring the World of Glycobiology

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Glycosylation in Health and Disease

Every cell, whether free-living or part of a multicellular organism, is decorated with a complex layer of sugars attached to surface macromolecules, such as proteins and lipids. These sugar-linked compounds, or glycoconjugates, are also found within and secreted by cells. Glycosylation, the enzymatic process that adds carbohydrates to biological molecules, enhances protein and lipid diversity, affects protein folding and stability, and is critical for a variety of biological processes, including cell communication and adhesion.

The role of glycosylation cannot be overstated. Researchers estimate that the mammalian glycome—the entire complement of sugars in an organism—consists of hundreds to thousands of structures (1). Glycobiologists dedicate their research to understanding the effects that these sugar additions have on cellular functions. While studies of rare genetic disorders affecting glycosylation provided the first clues of the process' importance, researchers now appreciate the effects of aberrant glycosylation on myriad disease states (2).

Cancer

Glycosylation pathway dysregulation is a hallmark of many cancers and often associates with poor prognosis. Incomplete synthesis of complex sugar chains is customary in early cancer stages, while de novo synthesis of neoantigen carbohydrate complexes on proteins is more common in advanced stages (3). Aberrantly-glycosylated proteins and the expression of certain glycosylation-specific genes can modulate cancer progression by regulating tumor proliferation, invasion, metastasis, angiogenesis, and treatment resistance (4). Additionally, factors in the tumor microenvironment (TME) can combine with glycosylation to promote tumor cell survival (3). The TME provides survival signals that encourage cancer cells to evade cell death. Glycosylation pathways also have roles in programmed cell death, such as obstructing ligand-receptor interactions and ligand secretion from effector cells in ways that protect against apoptosis and enhance cancer cell survival.

Neuroscience

Although it is the most common form of dementia, researchers still have a poor understanding of Alzheimer's disease (AD). While scientists know many of its clinical features, such as amyloid plaques in the brain, the underlying mechanisms that lead to disease initiation and progression remain unidentified. Patients with AD present with altered glycosylation profiles, and most known AD-related proteins are either glycosylated or regulate the glycosylation of other molecules (5). One example is amyloid precursor protein (APP), which can form toxic amyloid plaques depending on how it is processed. APP has numerous glycosylation sites, and researchers found that adding a particular sugar changed how the protein trafficks in cells. This sugar modification drives APP processing to its non-toxic form by increasing its localization to the plasma membrane (6). Enhancing this beneficial glycosylation event may present a therapeutic strategy for AD.

Infection

Throughout the COVID-19 pandemic, researchers have extensively studied the extracellular SARS-CoV-2 spike protein, which mediates attachment and entry into host cells via interactions with the angiotensin-converting enzyme 2 (ACE2) receptor. The SARS-CoV-2 spike protein is heavily glycosylated, and researchers suspect that this contributes to its enhanced receptor affinity compared to the related SARS-CoV protein. To determine the spike protein's glycosylation profile and better understand the sugars' roles in attachment and entry, scientists performed a glycopeptide analysis and identified a novel glycopeptide unique to SARS-CoV-2 near the furin cleavage site (7). Another research team found that glycosylation near this site decreased furin cleavage, which reduced the virus' infectivity in cell culture (8). A mutation found in both alpha and delta variants decreased this specific glycosylation event, which increased cleavage and hallmarks of viral infection.

Immunity

Glycosylation influences the innate and adaptive immune systems by adding sugars to most immune cell receptors. In particular, this process has huge implications for T-cell development and activity (9). For example, glycans on T-cell receptors play an important role in the proteins' affinities for their binding partners, major histocompatibility complex-antigen (MHC-antigen) complexes. Additionally, glycan motifs are important in autoimmune responses because they help T cells distinguish between self and non-self antigens. Abnormal levels of glycans associate with exacerbated immune responses in mouse models, including increased T-cell activation in hyperimmune responses (10,11).

Please see references on page 7.

Sweetening Up Biological Molecules

Sugars add structural and functional diversity to the macromolecules that they decorate, instilling new properties based on their chain length, branch structure, composition, and binding sites (1). Due to the seemingly endless number of possible sugar modifications and cellular processes that they affect, glycobiology may appear to be a daunting field to master. Understanding what these modifications are and how they are made is a big first step.

Decorating Macromolecules

Glycosylation is the enzymatic conjugation of a sugar with a functional group on a macromolecule, such as a protein or lipid, to form a glycoconjugate. Glycosyltransferase enzymes fuel these reactions by forming glycosidic linkages. These linkages covalently bond carbohydrates to each other or to macromolecules, forming glycan sugar chains that extend from the molecule's surface.

Glycosyltransferases have strict specificity toward their acceptor (macromolecule) and donor (sugar) molecules. Modifications of glycosyltransferase expression control cellular glycosylation events and indicate disease when dysregulated (2). Equally important for maintaining proper sugar modifications are glycosidases, which perform the reverse reaction: removing sugars by breaking glycosidic bonds through hydrolysis reactions.

A plethora of sugars can decorate proteins and lipids, from simple monosaccharide and oligosaccharide building blocks to long polysaccharide chains. The monosaccharides involved in glycosylation are often nucleotide sugars—energetic forms of monosaccharides that commonly contain a diphosphate group necessary for forming glycosidic bonds.

Protein and lipid glycosylation occurs through various mechanisms, typically co- or post-translationally in the endoplasmic reticulum (ER) or Golgi apparatus (GA), although some reactions can also occur in the cytoplasm (3). Simple sugars such as N-acetylglucosamine (GlcNAc), N-acetylgalactosamine (GalNAc), galactose, fucose, or mannose form the initial attachments to macromolecule functional groups, such as amines (-NH3) or hydroxyls (-OH).

Glycosylation Alphabet Soup

The two most common forms of glycosylation in eukaryotes are N- and O-glycosylation, where names refer to the atom to which carbohydrates link. N-glycosylation takes place in both the ER and GA. In the case of proteins, a glycosyltransferase adds a complex glycan with GlcNAc as its base to the nitrogen atom of an asparagine (Asn) that is part of a specific amino acid motif within the protein (Asn-X-Serine/Threonine). Once linked to its protein, it is further trimmed and modified to form linear or branched structures as the glycoconjugate journeys through the remaining ER and GA.

O-glycosylation initiates with the addition of a monosaccharide (commonly GlcNAc or GalNAc) to an oxygen atom. On proteins, O-glycosylation occurs on serine or threonine residues. This process commonly occurs in the GA as a series of reactions builds diverse glycans in a step-wise fashion. Secreted and extracellular glycoproteins are typically formed in the GA with O-glycan chains, while intracellular proteins have single GlcNAc sugars added through reactions in the cytoplasm. In the case of both N- and O-glycosylation, which modifications occur depends on the enzymes that a macromolecule encounters on its travels through the cell. Therefore, the enzymes can add different sugars to the same protein or lipid, producing distinct glycoforms with different properties.

Other minor forms of glycosylation include C-mannosylation—the addition of mannose to a tryptophan in a particular amino acid motif—and phosphoserine glycosylation, where glycans bind to serine residues via phosphodiester bonds. Finally, in glypiation, a glycan chain acts as a link between a protein and a phospholipid.

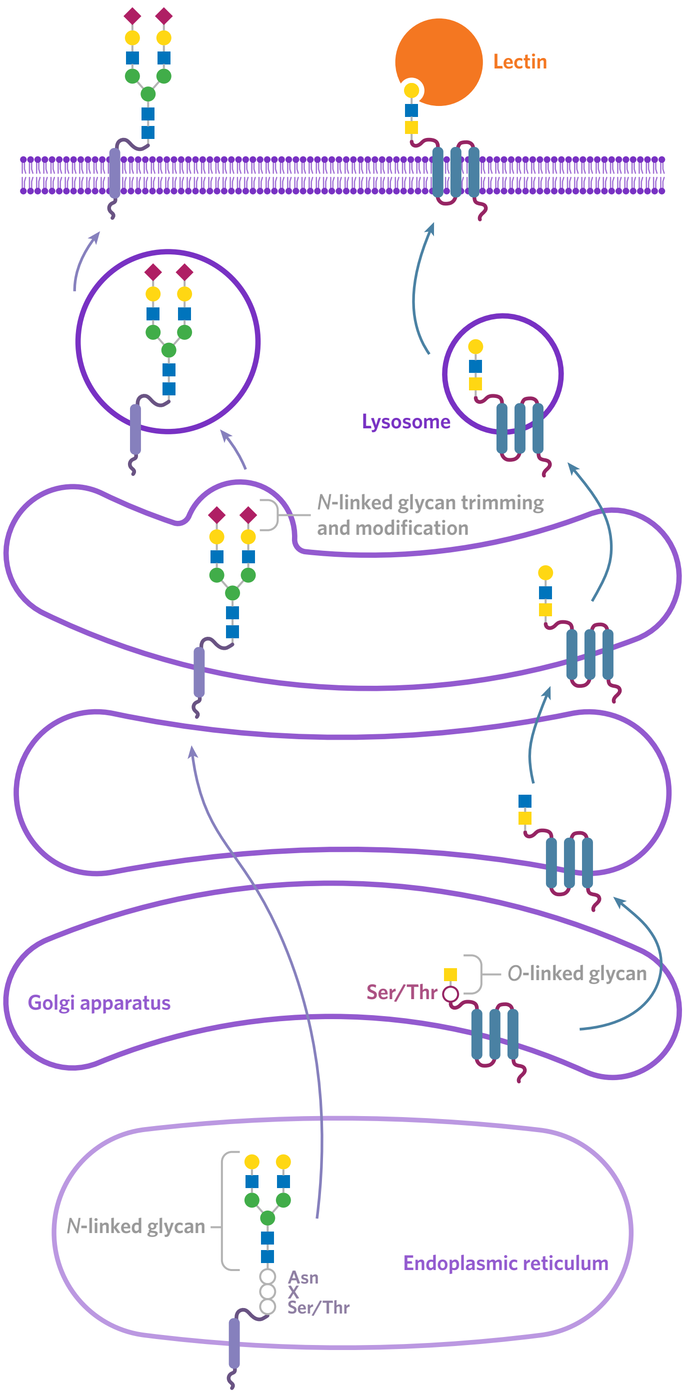
Introducing Lectins

Lectins are proteins that bind to glycoconjugates via interactions between the ends of glycan chains and lectin carbohydrate recognition domains. These contacts facilitate communication between cells that have extracellular glycoconjugates and lectins, leading to a variety of cellular events, including immune response activation and the regulation of cell adhesion and migration (4). Practically, glycobiologists use lectin binding properties as tools to study glycoconjugates. Because lectins recognize sugar structures, they can distinguish glycans with identical sugar compositions that are structurally different. A lectin's affinity for its carbohydrate binding partner varies upon even slight changes to the glycan structure, which makes them specific enough for sensitive laboratory assays, such as histology, flow cytometry, affinity chromatography, enzyme assays, and enzyme-linked immunosorbent assays (ELISAs).

Please see references on page 7.

Spotlight on Glycosylation

Glycosylation is a complex, multi-step process that adds diversity to numerous biological molecules, such as proteins and lipids. By affecting a molecule's stability and function, these modifications play large roles in normal cellular processes and many diseases.



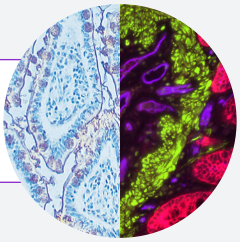
■ GlcNAc ● Galactose ■ GalNAc ● Mannose ◆ Sialic acid

Ser: Serine | Thr: Threonine | Asn: Asparagine | X: any amino acid

Lectins in the Laboratory

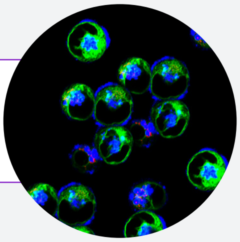
Due to their exceptional glycan-binding properties, lectins aid researchers in detecting glycoconjugates in a variety of ways.

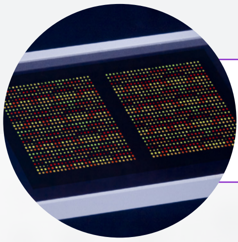
Histology
(Immunohistochemistry and Immunofluorescence)



ELISA
(Enzyme-Linked Immunosorbent Assay)

Flow Cytometry





Microarray

Lectin Applications in Glycobiology: Techniques and Research

In addition to their cellular functions, lectins are useful in the laboratory; researchers utilize their carbohydrate-binding ability in many ways. From early blood typing experiments to current detection and quantification assays, lectins are powerful research tools.

Widely distributed throughout nature, lectins were first discovered more than 130 years ago in plants and used for their ability to clump, or agglutinate, red blood cells (RBCs) (1). In the decades that followed, researchers began to uncover clues to their function. When scientists noticed that sucrose inhibited the clumping activity of the plant hemagglutinin concanavalin A, they began to appreciate its sugar-binding abilities. Further work demonstrated that different hemagglutinins bind distinct glycans on the RBC surface. This led to their use in early ABO blood group research as markers of blood type identity. Because they distinguish blood types, scientists formally named these biological molecules lectins, after the Latin verb legere meaning "to choose." Since then, researchers have used lectins to detect glycoconjugates of interest.

A Perfect Pairing

One quick and streamlined approach for identifying glycans is lectin-based enzyme-linked immunosorbent assays (ELISAs). In this technique, researchers attach proteins of interest to microtiter plate wells and apply biotinylated lectins to identify specific sugars in the samples (2). Researchers have used lectin ELISAs to identify glycans that act as disease biomarkers for cancer and infectious diseases (3,4).

Fluorescence-conjugated lectin reagents are useful tools for classifying cell subtypes via flow cytometry based on surface glycoconjugates. Researchers utilized this technique while studying different glycoforms of the heavily-glycosylated SARS-CoV-2 spike protein and ACE2 receptor (5). Using lectin-based flow cytometry, the researchers developed isogenic cell lines expressing different glycosylated forms of the spike protein and ACE2 and assessed their ability to bind. Additionally, they developed pseudoviruses that expressed various spike protein glycoforms to determine the effect of glycosylation on viral cell entry. The research team found that blocking the synthesis of certain glycoforms decreased viral entry, which suggests that chemical inhibitors of glycosylation may be useful COVID-19 treatments.

Researchers also use lectins in microarrays to probe the presence of certain glycoconjugates within a sample. In one example, researchers tested drug-resistant ovarian cancer cell lines with a lectin microarray (6). Drug resistance is common in ovarian cancer, and aberrant glycosylation is involved in this process. Using a lectin array, the researchers compared the glycosylation patterns of sensitive and resistant cancer cell lines. They confirmed the presence of notable glycoconjugates by western blotting with biotinylated lectins. Glycans specific to the resistant cell line may serve as drug resistance biomarkers to help clinicians determine patient prognosis and identify suitable chemotherapies.

Viewing the Glycome

In histology experiments, researchers visualize whole-cell glycosylation patterns with fluorophore- or biotin-conjugated lectins. One research group investigated how cancer cells maintain cell surface glycosylation, which contributes to cancer progression under nutrient deprivation and aberrant metabolism. The researchers used fluorescently-labeled lectins in a number of experiments, including confocal microscopy of cancer cells exposed to sialic acid (7). The added sugar contributed to protein glycosylation by enhancing sialyltransferase expression. These findings suggest that cancer cells may scavenge sialic acid from the microenvironment to overcome nutrient deprivation and maintain surface glycosylation.

In an effort to understand the transmission of bird flu to humans, another team used biotinylated lectins to probe human and pig tissues for avian influenza virus (AIV) attachment (8). To transmit to humans, AIV must adapt to bind to different glycosylated host receptors in the respiratory tract. This adaptation was previously thought to require passage through another mammalian host, such as pigs. The researchers detected AIV's preferred sugar in human tissues using lectin probes and found that the virus attached more abundantly to the human respiratory tract than to that of pigs, which suggests that AIV does not require passage through pigs to infect human hosts.

Please see references on page 7.

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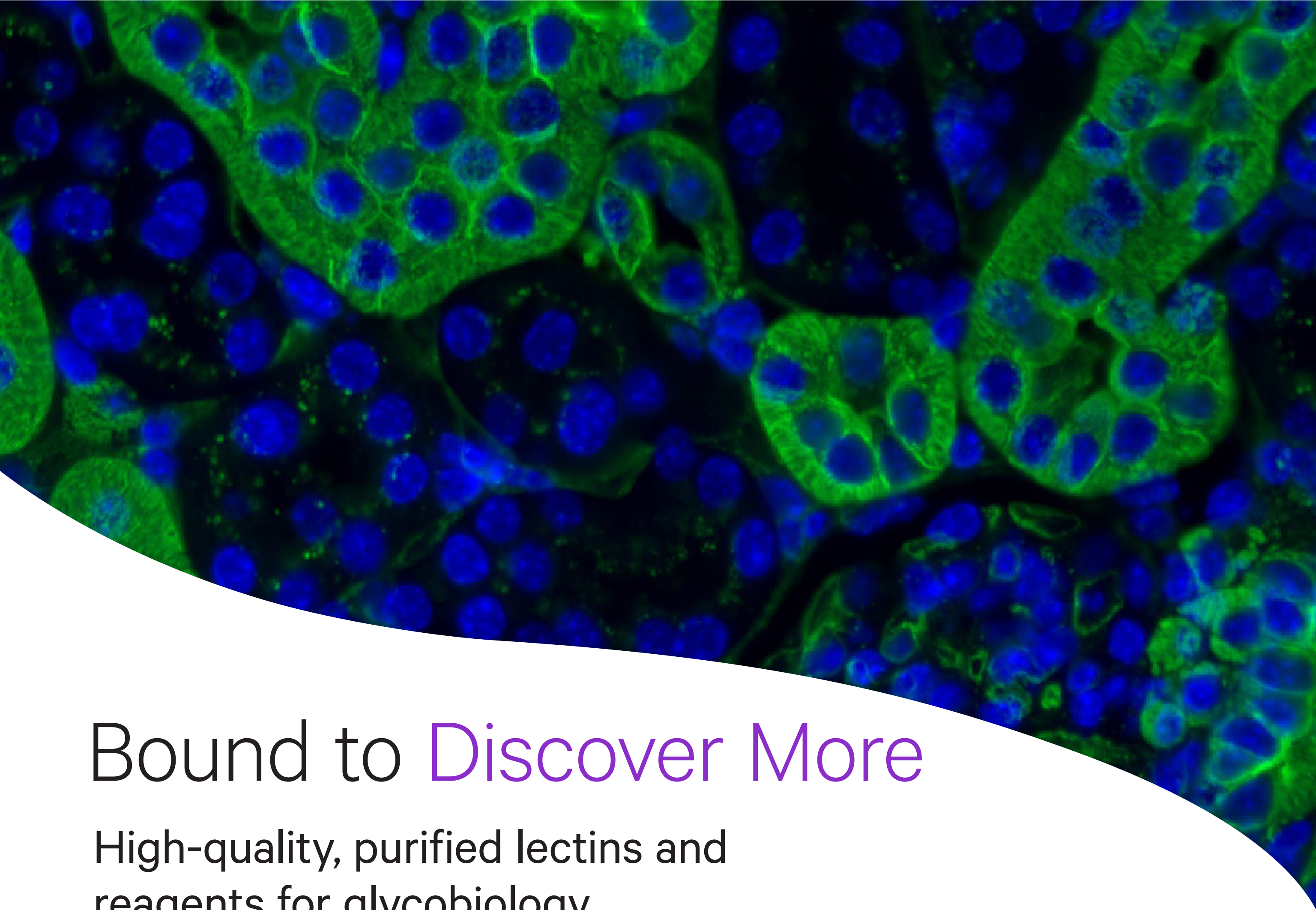

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High-quality, purified lectins and reagents for glycobiology

Lectins are the key to help you profile, characterize, and capture complex glycans in biological systems or leverage functional assays to ask questions that were previously beyond reach.

Specifically, lectins can be used in glycobiology to:

- ✓ Separate oligosaccharides, even those with identical sugar compositions
- ✓ Discriminate between oligosaccharide structures
- ✓ Isolate a specific glycoconjugate, cell, or virus from a mixture

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