1. Keywords and Terms

Glycobiology, oligosaccharides, N-linked glycans, O-linked glycans, glycosyltransferases, glucose, sialic acid, sialyic lewis X

2. Lecture Notes

Glycosylation is a post-translational modification. The complexity of an organism is not entirely encoded by its genome. Much of the complexity and molecular functions of an organism results from post-translational modifications. Post-translational modifications are the covalent addition of molecules to a protein after translation from RNA and are not encoded in the genome. One such example of post-translational modification is glycosylation, or the addition of one or more sugar molecules to a
protein, often serving to alter its function. Glycosylation is the most complex form of post-translational modification. Lipids, in addition to proteins, can also be glycosylated. The majority of glycoproteins and glycolipids are found on the cell surface where they can interact with other cells or pathogens.

Oligosaccharides can be highly complex with elaborate branched structures. The totality of glycans or sugar molecules produced by a cell is called the glycome. The glycome is highly dynamic and can change depending on the physiology of the cell. For example, a cell will change its glycan structures when it changes from an embryonic cell to a differentiated cell or when a cell goes from a healthy state to a diseased state. Additionally, different organisms have different glycans. In vertebrates, glycans are composed of nine basic sugar molecules: glucose (Glc), galactose (Gal), mannose (Man), N-acetylglicosamine (GlcNAc), glucuronic acid (GlcUA or GlcA), N-acetylglactosamine (GalNAc), sialic acid (Sia), fucose (Fuc), and xylose (Xyl). From the monosaccharide or single sugar building blocks a very large number of complex glycans are synthesized. By connecting two monosaccharides together through a glycosidic bond, a disaccharide is formed. For example, the connection of glucose to galactose forms lactose, a common disaccharide found in cow’s milk. The wide structural diversity of glycans arises from differing regiochemistry and stereochemistry of the linkages between monosaccharides. Regiochemistry denotes which hydroxyl group on a given monosaccharide participates in the glycosidic bond with the adjacent sugar, and stereochemistry refers to the orientation of the linkage (either α or β). These two factors are often critical parameters for correct recognition of the glycan by glycan-binding proteins. Using lactose as an example the galactose is connected by its hydroxyl group at position 1 to the position 4 hydroxyl group on glucose in a β orientation. This glycosyl bond would be called a β (1→4) linkage; the β denotes the stereochemical orientation and 1→4 contains the regiochemical information. Disaccharides can be further elaborated to form oligosaccharides or polysaccharides. Oligosaccharides and polysaccharides are large glycan molecules containing many monosaccharide subunits in linear or branched structures. Oligosaccharides are described as having a reducing and a non-reducing end analogous to DNA’s 5-prime and 3-prime ends or a protein’s N-terminus and C-terminus. The reducing end of a glycan is the point where the glycan is attached to either a protein or lipid. The non-reducing ends are where oligosaccharides are further elaborated.

Glycoproteins are synthesized in the ER and Golgi apparatus by glycosyltransferases. Oligosaccharides are classified by the amino acid to which they
are attached. **N-linked glycans** are attached to proteins on asparagine residues and **O-linked glycans** are attached to either a serine or threonine residue of a protein. In vertebrates, the pentasaccharide glycan core of N-linked glycans is highly conserved. By contrast, O-linked glycans only share a single GalNAc residue at the reducing position. From the conserved core residue(s), the glycan is further elaborated to have one or more antennae, or branched chains. In the case of N-linked glycans, the core glycan is attached to the protein in the endoplasmic reticulum (ER) and further elaborated into highly complex structures in the ER and Golgi compartments before the glycoproteins are trafficked to the plasma membrane or other organelar membranes within the cell, or secreted into the extracellular medium. **Glycosyltransferases** are enzymes in the ER and Golgi apparatus that transfer the **glycosyl donors**, or activated monosaccharides, to the growing glycan. The monosaccharides are activated by attachment to a nucleoside via a monophosphate or diphosphate bridge.

The human blood groups are determined by cell surface glycans. One of the most important discoveries of modern medicine was the determination of blood type differences. Each blood type is defined by a particular glycan structure on the cell surface of red blood cells. Blood group O is defined by an O-linked trisaccharide. People with blood type A have a glycosyltransferase that adds a GalNAc residue to the non-reducing end of the type O trisaccharide. People with blood type B have a different glycosyltransferase that adds Gal in the same stereochemistry and regiochemistry to the type O trisaccharide. The human immune system can detect the difference between the GalNAc and Gal on the cell surface. People with blood type AB have both of these glycosyltransferases and therefore display a mixture of oligosaccharides that contain both Gal and GalNAc on their red blood cell surfaces.

The influenza virus uses host glycans for infection. Viruses require the use of host machinery to replicate and propagate the viral particles. In order to do this, the virus needs to get inside the cell. The influenza virus is enclosed in a membrane containing two glycoproteins: hemagglutinin (H) and neuraminidase (N). Hemagglutinin is a receptor that binds sialic acid (Sia) on the cell surface. This allows the virus to dock on the cell membrane and fuse its membrane with that of the host cell. Once the virus has entered the cell, it uses host machinery to replicate itself. In order to continue the infection, virus particles must bud off of the host membrane. To do this, it uses another protein in its membrane, neuraminidase, to cleave Sia bound to hemagglutinin off the cell surface, allowing the virus particle to float away to infect other cells. A number of neuraminidase inhibitors have been developed to stop the propagation of the virus inside the host. The chemical structures of Relenza and Tamiflu, two commercial antiflu
thereputics, both mimic the transition state of neuraminidase-bound Sia residue during the cleavage reaction. Basic research of the influenza virus has revealed the important functions of glycans during infection and enabled development of therapeutics to combat infections.

*Tissue inflammation causes changes in the epithelial glycan structures which are sensed by circulating leukocytes.* In the initial stages of inflammation, endothelial cells, which are cells that line your blood vessels, undergo a physiological change that results in production of a different set of glycans on their cell surfaces as well as the expression of two receptors, P-selectin and E-selectin, that bind to glycans on the cell surface of leukocytes, circulating white blood cells. Additionally, leukocytes express cell-surface receptors termed L-selectin that are specific to a unique glycan, sialyl Lewis X, which is presented on inflamed epithelial cells. The selectins reversibly bind to glycans on the cell surface, causing the leukocyte to attach to the endothelial cell in a process termed “leukocyte rolling” and come to a stop on the surface of blood vessels. The leukocytes can then migrate to the surrounding tissue in a process called extravasation. Inflammation is characteristic of many diseases such as rheumatoid arthritis, asthma, type 1 diabetes and many more. Inhibitors of selectin-mediated cell adhesion could function as broad-spectrum anti-inflammatory drugs. Unfortunately, the selectins have low affinity for sialyl Lewis X. To increase the binding ability, cells produce large brush-like glycoproteins that contain many of copies of sialyl Lewis X for the selectin to bind. Selectins also contain multiple glycan-binding domains per protein molecule. Thus, glycan binding by selectins results in a large multivalent complex. Just as multivalent ligands are employed in nature for cell-cell attachment, multivalent inhibitors are likely to be better drug candidates than traditional monovalent, small-molecule inhibitors. One example of a multivalent inhibitor is a liposome, an artificial spherical structure composed of a lipid bilayer surrounding a drop of liquid meant to mimic a cell. Liposomes can be synthesized to display thousands of sugars attached to the surface to mimic the multivalent ligands. These can out-compete the natural ligands to inhibit inflammation.

**Conclusions:** Glycans are the most complex post-translational modification and are primarily found on the surface of cell membranes. The glycome changes during physiological transformations. Both normal and disease processes are mediated by changes in the glycans which are important in cell-cell and cell-pathogen interactions. Glycobiology has led to an understanding of cellular processes as well as a number of important therapeutics.
3. Review Questions

1. Glycosylation is the most complex form of
   a. Proteins
   b. Cell surface receptors
   c. Post-translational modification
   d. Virus

2. Polysaccharides are synthesized by
   a. Hemagglutinin
   b. Selectins
   c. Liposomes
   d. Glycosyltransferases

3. In a trisaccharide, what is the bond between each sugar called?

4. What is a glycosyl donor?

5. How do people with blood type O differ from people with blood types A, B, or AB?

6. What is the function of hemagglutinin in influenza infection?

7. How do Relenza or Tamiflu inhibit influenza virus propagation?

8. True or False: It is normal for the lymph node to collect leukocytes

9. What is multivalency?

4. Answers to Review Questions

1. c. Post-translational modification
2. d. Glycosyltransferases

3. A glycosyl bond.

4. A monosaccharide activated with a nucleotide diphosphate.

5. People with blood type A have galactosamine attached to the type O trisaccharide; individuals with blood type B have galactose attached to the type O trisaccharide; people with blood type AB have a mixture of A and B tetrasaccharides.

6. Hemagglutinin binds to sialic acid on the host cell surface to fuse the viral membrane with the host cell.

7. Relenza and Tamiflu inhibit the neuraminidase enzyme from cleaving sialic acid residues from the host cell surface, causing the budding viral particles to stay stuck to the host cell due to hemagglutinin’s binding of sialic acids.

8. True

9. Multivalency is when multiple receptors bind multiple ligands.

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**5. Discussion Questions**

1. What are some conditions or situations that you think might cause a cell to change the glycans on its surface?

2. We have seen a couple examples of how cells can change the glycans on their cell surfaces; why might this be important?

3. Comparing the structures of the nine monosaccharides from vertebrates, how are they similar and how are they different? Drawing out glucose, how can you change it to become galactose, mannose, N-acetylglucosamine, or xylose?

4. How might you develop a therapeutic to inhibit hemagglutinin from performing its natural function?
6. Answers to Discussion Questions

5. Cells change their cell-surface glycans during physiological change which includes inflammation, cancer, stem cell differentiation, bacterial or viral infection and many more.

6. A cell changes the glycans on its cell surface to communicate to other cells that it is undergoing a physiological change. If this change is during the course of infection, it can indicate to surrounding cells to defend themselves or to recruit the immune system for help with fighting the infection.

7. Glucose can be converted to galactose by changing the stereochemistry at position 4 from equatorial to axial. Glucose to mannose: position 2 equatorial to axial. Glucose to N-acetylglucosamine: change the hydroxyl to a nitrogen atom with an acetyl group. Glucose to xylose: remove the methylene and hydroxyl from position 6, replacing them with a hydrogen atom.

8. To inhibit hemagglutinin, a liposome containing many sialic acids could be used to bind all the viral particles.

7. Explain or Teach These Concepts to a Friend

1. Explain how stereochemistry and regiochemistry describe the glycosidic bond.

2. Explain how glycans are biosynthesized.

3. Describe sialic acid and its role in the lifecycle of influenza.

4. Explain how understanding glycobiology has lead to the development of therapeutics.

5. Explain how glycans mediate cell-cell interactions.
8. Research the Literature on Your Own

1. Research the importance of glycoproteins in the Human Immunodeficiency Virus or other viruses (search viral glycoproteins).

2. Investigate mucins and how they can be used for cancer diagnosis (search mucins cancer)