

Giving cells a new sugar-coating

Mark Howarth & Alice Y Ting

Looked at from the outside of the cell, proteins are often hidden behind a forest of sugar chains. Using a sugar analog to introduce thiols onto the tips of the branches of this forest alters cell attachment and has unexpected consequences for cell differentiation.

Any binding event between a ligand and its cell surface receptor, between one cell and another, or between the cell and its surroundings must be considered in the context of the dense thicket of oligosaccharides on the surface of mammalian cells. On page 149 of this issue, Sampathkumar *et al.* describe using a small-molecule sugar analog to redecorate the cell surface with thiol-bearing sugars¹. They find that this sugar analog changes the cell's anchorage to the extracellular matrix and may also promote differentiation of stem cells.

There has been an explosion in our understanding of glycosylation in the last decade, founded on the ability to decode the structure of complex branched oligosaccharides and to synthesize them chemically². We are now very skilled at engineering the expression of proteins at the cell surface, but the remodeling of glycosylation has been a much greater struggle. Pioneering work to engineer surface sugars showed that it was possible to tap into the cell's sialic acid pathway by supplying modified versions of the sialic acid precursor *N*-acetyl-*D*-mannosamine (ManNAc)^{3,4}. This is a good place to start because ManNAc is present at low concentration in the cell, is uncharged, and is not metabolized to any significant degree into sugars other than sialic acid. ManNAc was previously modified with azide or ketone groups, which are not normally present on the cell surface and so can be uniquely targeted by chemical reactions⁴. This targeted sugar modification was used, for example, to convert a cell into a target for a viral infection⁵ or to attach DNA to the cell surface so that cell adhesion could be directed by DNA hybridization⁶.

Sampathkumar *et al.* made use of a thiol analog of ManNAc and investigated how it affects cell behavior¹. The first step is to sneak the analog into the cell by masking its hydroxyls with acetyl groups (acetylated thio-ManNAc,

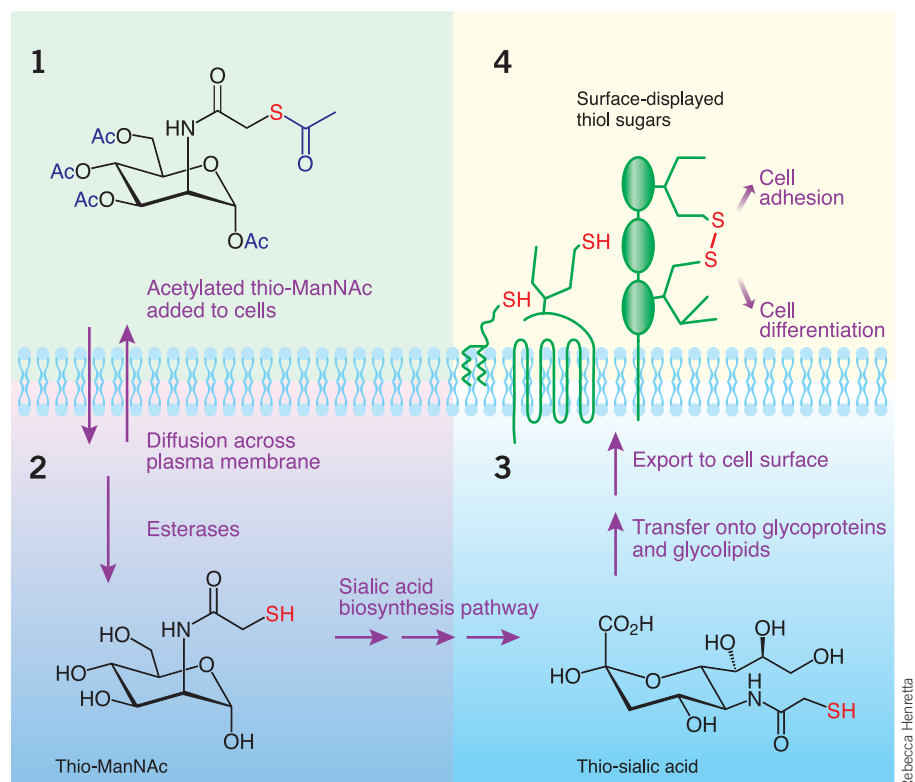


Figure 1 How an acetylated thiol sugar may be taken up by cells and redecorate the cell-surface with thiol groups.

Fig. 1), to help it diffuse through the plasma membrane. In the cytosol, esterases remove these acetyl groups to give thio-ManNAc (**Fig. 1**). Thio-ManNAc then infiltrates the pathway for sialic acid synthesis, which should convert it to a thiol analog of sialic acid (thiosialic acid, **Fig. 1**). The final step is to transfer the thio-sialic acid onto glycolipids or glycoproteins in the Golgi and to hitch a ride through the secretory pathway for display on the cell surface. In contrast to strategies for the introduction of unique chemical functionalities onto cell surface proteins^{7,8}, there will not be selectivity as to which proteins display the sugar analog, because more than 80% of all the sialic acid on surface glycoproteins can be replaced by analogs³.

What is the point of introducing a thiol analog of a sugar? The thiol group has

three characteristic reactivities—first, nucleophilic attack on acceptors such as maleimides and iodoacetamides; second, oxidation with other thiols to form disulfide bonds; and third, strong coordination of soft metals such as gold. Thiols are endogenously present in the cytosol, but in the secretory pathway and at the cell surface most thiols are oxidized to disulfide bonds. Thus when the authors incubated cells with acetylated thio-ManNAc, the number of thiols at the cell surface, detected by labeling with biotinylated maleimide, increased greatly. This indicated that thiol sugars were efficiently displayed on the cell surface. However, adding a reducing agent increased the exposure of surface thiols by about ten-fold, indicating that 90% of the thiol sugars had already formed disulfide bonds.

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How did the cells respond to their new coat? T cells treated with thio-ManNAc clustered markedly, presumably as a result of disulfide bond formation between thio-sialic acid residues on different cells. Human stem cells, on the other hand, did not cluster when treated with thio-ManNAc, but over two weeks they underwent changes in gene expression and morphology characteristic of neuronal differentiation. This effect echoes previous intriguing results with a ManNAc analog that differed only in the replacement of the acetyl with a propyl group: this ManNProp strongly stimulated T cells to divide and changed the response of oligodendrocytes to neurotransmitters³.

It will be fascinating to find out how the thiol sugar exerts its functional effects. Do the effects perhaps result from disulfide bond

cross-linking of proteins bearing thiol sugars, or from changes to the binding of proteins to sialic acids? Also, is the thiosialic acid the only derivative of acetylated thio-ManNAc to reach the cell surface? Glycobiology is undergoing dramatic progress, but the ability to perturb oligosaccharide function, rather than simply observe it, will be key to future advances in this field. Because sugars have such an important role in cell-cell communication, it will be crucial to find ways to control sugar structure in tissues and organisms⁹. This is highlighted by the promising reduction in metastasis caused by modulating the sialic acid structures of tumor cells¹⁰. That the sugar analog in this study had such notable effects on substrate adhesion and stem-cell fate suggests that the strategy of controlled re-engineering of the

cell's sugar coat still has many lessons and surprises in store for us.

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Watching single protons bind

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Using single-molecule biophysical studies in an ion channel, the protonation state of engineered basic amino acids was measured in real time, making it possible to calculate the pK_a s of the substituted residues and creating a unique, comprehensive dataset for theorists studying the effects of an electrostatic environment on integral membrane protein function.

Many integral membrane proteins contain ionizable amino acids in their transmembrane domains. To understand the functional role of these protonatable side chains, the factors that determine the ionization state and the spread of the electrical potential through the protein interior must be elucidated. Theorists have developed molecular models that attempt to describe the electrostatic environment inside a protein and explain the available experimental data¹. Previous experimental studies have been limited by the difficulty of determining the protonation state and pK_a of engineered ionizable residues. In a recent paper in *Nature*², Grosman and co-workers used single ion-channel current measurements as a real-time reporter for the protonation state of lysine and histidine residues substituted at each position in the channel-lining segment of the nicotinic

acetylcholine receptor (nAChR)². These data allowed them to calculate the pK_a values for each substituted residue and provided new information on the electrostatic microenvironment in and surrounding the ion channel.

Ion channels are polytopic membrane proteins that form water-filled pores through cell membranes and facilitate ion translocation at rates exceeding 10^7 ions per second. An important goal of ion channel studies has been to elucidate the physical and chemical mechanisms that determine the rate of ion translocation. Five homologous subunits, $2\alpha:1\beta:1\delta:1\epsilon$, assemble to form nAChRs (Fig. 1a). Each subunit contains four α -helical transmembrane segments (M1, M2, M3 and M4)^{3,4}. The five M2 helices form the channel lining in the 4-Å-resolution, closed-state channel structure and are separated from the lipid bilayer by an outer ring of helices formed by M1, M3 and M4 (Fig. 1b)⁵. Binding of acetylcholine to the extracellular domain induces rapid opening of a cation-selective channel⁶.

To probe the electrostatic environment in the open channel, Grosman and co-workers substituted lysines and histidines, one at a time, into 30 consecutive positions in and

flanking the δ -subunit M2 segment of nAChR and assayed the accessibility of the substituted residues to protons by single-ion channel conductance measurements². The fundamental assumption in these experiments was that protonation of a basic amino acid in the channel-lining segment would reduce the current passing through the channel, because of electrostatic repulsion between the fixed charge in or on the channel wall and the permeating cations (Fig. 1c). Three current levels were observed for residues where the duration of protonated and deprotonated intervals were longer than the 25- μ s time resolution of the single-channel patch-clamp recording apparatus: the largest current level corresponded to the deprotonated open state current, a subconductance level represented the protonated open state and the zero current level reflected nonconducting closed or desensitized states (Fig. 1d)². The extent to which protonated lysines reduced the single-channel current was inferred to be proportional to the residue's distance from the central channel axis, and the extent of current reduction showed an α -helical periodicity². The reduction in single-channel current was greatest at

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