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Complex *N*-glycans: the story of the "yellow brick road"

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Abstract The synthesis of complex asparagine-linked glycans (N-glycans) involves a multi-step process that starts with a five mannose N-glycan structure: [Manα1-6(Manα1-3)Manα1-6][Manα1-3]-R where R=Manβ1-4GlcNAcβ1-4GlcNAcβ1-Asn-protein. *N*-acetylglucosaminyltransferase I (GlcNAc-TI) first catalyzes addition of GlcNAc in β1-2 linkage to the Manα1-3-R terminus of the five-mannose structure. Mannosidase II then removes two Man residues exposing the Manα1-6 terminus that serves as a substrate for GlcNAc-T II and addition of a second GlcNAcβ1-2 residue. The resulting structure is the complex N-glycan: GlcNAcβ1-2Manα1-6(GlcNAcβ1-2Manα1-3)-R. This structure is the precursor to a large assortment of branched complex N-glycans involving four more *N*-acetylglucosaminyltransferases. This short review describes the experiments (done in the early 1970s) that led to the discovery of GlcNAc-TI and II.

Keywords Complex N-glycans · N-acetylglucosaminyltransferases · N-glycan branching · N-glycan core structure · Chinese hamster ovary (CHO) cells · Resistance to lectin cytotoxicity · *Phaseolus vulgaris* (L-PHA) · Cell surface glycosylation · lectin phytohemagglutinin · biological recognition signals

It all began in Hart House at the University of Toronto in the early 1970s. Hart House at that time was a place for male students to meet, eat and indulge in various athletic endeavors. Rest assured that this excellent facility has been open to both men and women for many years. On that fateful day, shortly after a swim in the Hart House pool, I had what turned out to be an epic talk with Lou Siminovitch. Lou was already at that time a highly renowned geneticist who had recently created the University of Toronto's first Department of Medical Genetics. Lou told me that a bright young post-doctoral student (Pamela Stanley) had joined his group and had isolated mutant Chinese hamster ovary (CHO) cells selected for resistance to the cytotoxic action of the lectin phytohemagglutinin from *Phaseolus vulgaris* (L-PHA).

Lectin cytotoxicity was known to follow the interaction of a lectin with carbohydrate moieties on the cell surface [1, 2]. Between 1971 and 1974, several groups had reported mutant cell lines resistant to the cytotoxic effects of various lectins [3–8]. Pamela had shown her CHO mutants (selected for resistance to L-PHA) to be resistant to other lectins that bound galactose. But they were hypersensitive to Con A [9] suggesting loss of terminal residues of membrane glycans. Gel electrophoretic analysis revealed a reduction in the molecular weight of many membrane proteins [10]. We therefore postulated that her mutant cells lacked either the galactosyltransferase (Gal-T) or *N*-acetylglucosaminyltransferase (GlcNAc-T) necessary to build the complex N-glycans attached to the cell surface glycoproteins. N-glycans are oligosaccharides attached to the protein by way of a GlcNAc-β1-Asn linkage. Mutant lines similar to Pamela's were reported between 1970 and 1975 by the groups of Kornfeld [11, 12], Osawa [13], and Hughes [14].

As early as the 1960s [15, 16] N-glycoproteins were shown to contain complex N-glycans with a characteristic trisaccharide (sialic acid-Gal-GlcNAc-) at the non-reducing ends. In 1974, Montreuil named these oligosaccharides 'antennae' to emphasize that a possible functional role for these terminal structures was the transmission of biological recognition signals [17]. At about the same time, Kobata

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reported the presence of the GlcNAcβ1-2-Man moiety on the N-glycan core structure [18, 19].

A fairly detailed description of our journey along "The Yellow Brick Road" of N-glycan branching has been published [20]. I will limit this relatively short article to my work with Lou Siminovitch and Pamela Stanley in the mid-1970s.

At the time of my Hart House meeting with Lou, my group had published more than a dozen papers on various glycosyltransferases. Lou therefore conscripted me to the project. The resulting collaboration turned out to be a major turning point in my scientific career. Lou is still as healthy and argumentative at 93 as he was in the 1970s! My wife Judy and I frequently get together with him for concerts.

Our collaboration resulted in two publications: Pamela did the genetic work in Lou's laboratory [21] and collaborated with Saroja Narasimhan on the enzyme work in our laboratory [21, 22]. Lou very graciously did not put his name on the author list of the second paper. I suspect that he felt it was beneath his dignity to put his name on a purely biochemical publication!

We found that the GlcNAc-T activities of extracts from several clones of PHA-resistant CHO cells, using several mannose-terminal glycoprotein acceptor substrates, varied from 4 % to 55 % relative to wild type CHO cells [21]. The absence of this GlcNAc-T activity resulted in defective addition of GlcNAc residues to the lectin-binding glycoproteins on the cell surface. No significant differences between lectin-resistant and wild-type cells were noted for the activities of sialyl-, fucosyl-, galactosyl- or mannosyltransferases [21].

We found a significantly high level of GlcNAc-T activity in our mutant cells (25 % to 55 % relative to wild type) using some of our mannose-terminal glycoprotein acceptor substrates. This finding suggested that wild type CHO cells may possess at least two GlcNAc-T enzymes that add GlcNAc to the mannose termini of the *N*-glycan core and that only one of these GlcNAc-T enzymes was absent in our PHA-resistant CHO cells. We purified Immunoglobulin G from the serum of a rather special multiple myeloma patient, digested the protein with Pronase and prepared bi-antennary glycopeptides with a large variety of terminal sugars [22]. We then used these glycopeptides as acceptor substrates for the assay of GlcNAc-T in our wild type and mutant CHO cells. We found PHA-resistant CHO cells showed no significant GlcNAc-T activity with glycopeptide acceptor substrates in which both antennae terminated in a mannose residue but showed significant GlcNAc-T activity with a biantennary acceptor in which the Manα1-3Manβ1-4- antenna but not the Manα1-6Manβ1-4- antenna was terminated by a GlcNAcβ1-2 residue. We had discovered that at least two GlcNAc-T enzymes were required for the conversion of the core N-glycan structure, Manα1-6(Manα1-3)Manβ1-4R, to complex N-glycans. In subsequent work by the groups of Pamela Stanley and Stuart Kornfeld [23–25], it was shown that under physiological conditions, GlcNAc-TI acts on the $Man_5GlcNAc_2$ protein-bound moiety (M5 to 2M5hy, Figs. 1, 2 and 3) thereby allowing removal of two Man residues by mannosidase II [26] followed by the action of GlcNAc-TII (Figs. 1, 2 and 3).

Our laboratory subsequently published a series of papers on "The Control of Glycoprotein Synthesis" [27–37]. Details of these studies and those of other laboratories cannot be described in this short review. However, "The Yellow Brick Road" [20] that resulted from these studies is shown in Fig. 2.

The functions that all these complex N-glycans (Fig. 2) play in biology have been the subject of much research and debate. N-glycosylation is one of the most common protein post-translational modifications and nearly half of all known proteins in eukaryotes are probably N-glycosylated. Since GlcNAcT-I is essential for the formation of these N-glycans, several studies have been published on the effects of removing the GlcNAcT-I gene. Cultured cells and plants lacking GlcNAcT-I appear normal but mammals have an absolute

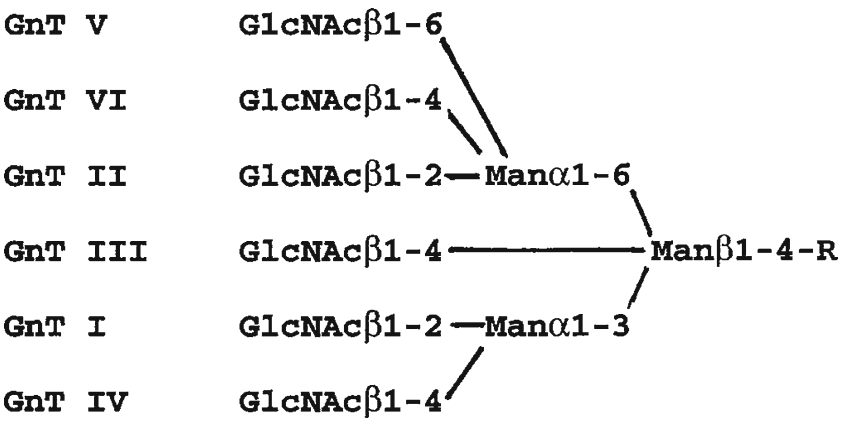

Fig. 1 Sites of action of N-acetylglucosaminyltransferases (GnT) I to VI on protein-bound N-glycans. R=GlcNAcβ1-4GlcNAc-Asn-protein. GnT V was first described in 1982 by R.D. Cummings, I.S. Trowbridge and S. Kornfeld in studies using a PHA-resistant mouse lymphoma cell line (J.Biol.Chem. 1982. 257, 13421–13427)

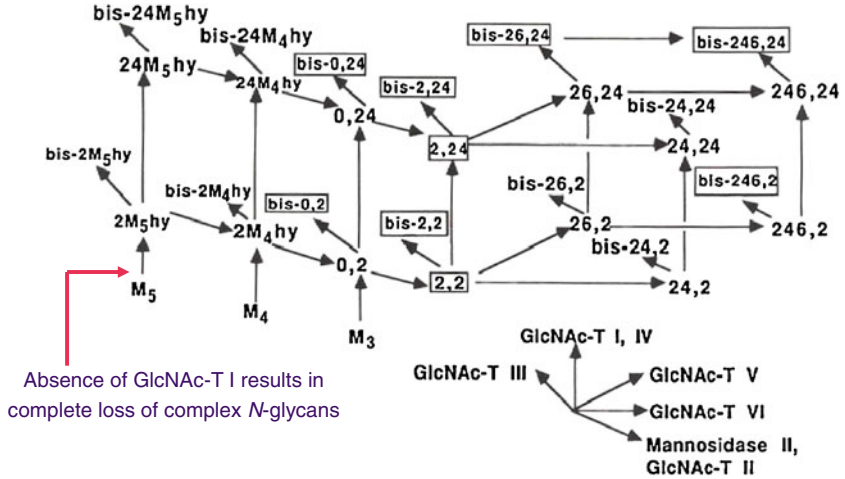

Fig. 2 This figure shows the "Yellow Brick Road" for complex N-glycan synthesis (Schachter, H. Glycobiology, vol 1 (5), pgs.453–461, 1991). The name of the figure refers to the several different roads that Dorothy and her friends blundered into before they arrived at the Emerald City. Our chemical road is equally complex. The scheme on the lower right section of the figure shows the enzymes (GlcNAc-Ts and mannosidase II) that act at the various arrows in the Figure. The road starts on the lower left with a glycosyltransferase that we named GlcNAc-T I. This enzyme adds a GlcNAc residue in β1-2 linkage to the Manα3 arm of an N-glycan carrying 5 mannose residues (M_5, see Fig. 3 for explanation of abbreviated structure names) to form the structure 2M5hy. The term "hy" refers to the term "hybrid N-glycan" because these structures contain only Man residues on the Manα1-6 arm and only GlcNAc residues on the Manα1-3 arm. The term "bis" (*e.g.*, in the structure bis-2M5hy, Fig. 3) refers to structures with a bisecting GlcNAc, *i.e.*, a GlcNAc attached to the Manβ1,4GlcNAcβ1,4GlcNAc-Asn moiety of the glycoprotein. The addition of the bisecting GlcNAc is catalyzed by GlcNAc-T III. The nomenclature for non-hybrid structures does not have an "M" because the two arms of the 3-Man core structure do not have any Man residues attached to them. The 3-Man core may be unsubstituted or may have one or more GlcNAc residues attached. For example, the 3-Man core of the bis-2,24 structure (Fig. 3) has a bisecting GlcNAc (bis) attached to the Manβ1-4GlcNAcβ1-4GlcNAc-Asn moiety, a GlcNAc attached in β2 linkage to the Manα1-6 arm, and two GlcNAc residues attached in β2 and β4 linkages to the Manα1,3 arm

requirement for this enzyme and die during early embryogenesis [38–40]. Null mutations in *Drosophila melanogaster* GlcNAcT-I produce defects in locomotion and a reduced lifespan [41]. *Caenorhabditis elegans* has three distinct GlcNAcT-I genes and deletion of all three genes appears to have no effect on these organisms; however, we have obtained evidence suggesting that GlcNAcT-I dependent N-glycans may be involved in the response of the organism to bacterial pathogens [42].

Taniguchi [43] has suggested that changes in oligosaccharide structures are associated with many physiological and pathological events, including cell growth, migration, differentiation, tumor invasion, host-pathogen interactions, cell trafficking, and transmembrane signaling. Dennis [44, 45] has published several papers based on the concept that "metabolite availability to the hexosamine and Golgi N-glycosylation pathways exerts control over the assembly of macromolecular complexes on the cell surface and, in this capacity, acts upstream of signaling and gene expression" and "most transmembrane receptors and solute transporters are glycoproteins, and the Asn (N)-linked oligosaccharides (N-glycans) can bind animal lectins, forming multivalent lattices or microdomains that regulate glycoprotein mobility in the plane of membrane." However, much work remains to be done on complex N-glycan function.

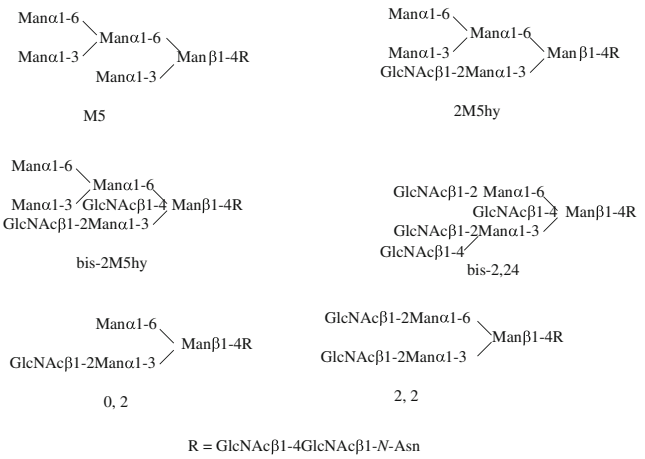

Fig. 3 Abbreviated names of N-glycan structures

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