



The search for glycan function: Fucosylation of the TGF- β 1 receptor is required for receptor activation

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Interest in glycobiology was for many years restricted by a lack of information on the functions of glycans (1). The diversity of linkages and branching patterns present in glycans confers on them the ability to carry a great deal of information in very compact structures (2). Glycans conjugated to proteins by β -linkage of *N*-acetylglucosamine (GlcNAc) to the amido group of Asn (*N*-glycans) and by α 1-*O*-glycosyl linkage of *N*-acetylgalactosamine (GalNAc), mannose (Man), and fucose (Fuc) to Ser and Thr (*O*-glycans) are primarily membrane-bound or extracellular. These and other considerations suggested that *N*- and *O*-glycans play essential roles in the interactions of cells with their cellular and fluid environments. Over the past 10 years, studies on mice (3–6) and humans (6–12) with mutations that result in defective protein *N*- and *O*-glycosylation have proved this hypothesis to be correct. To date, at least 30 mutant mice and 23 human congenital diseases have been described with null mutations in genes involved in protein *N*- and *O*-glycosylation (6). In a relatively small number of these mutant mice and humans it has been possible to construct a reasonably accurate scenario of the molecular mechanisms involved in the production of the phenotype because it has been possible to identify a specific protein target of the mutant enzyme and study the role of the glycan in the function of that target protein. The article by Wang *et al.* (13) in this issue of PNAS is an important example of such a situation. However, for most of the mutant mice and humans, the mutant enzyme alters the glycosylation of a large number of different glycoproteins, and it is not possible to deduce the precise molecular mechanisms responsible for the phenotype.

Wang *et al.* (13) generated mice with null mutations in the *fut8* gene encoding GDP-L-Fuc:*N*-acetyl- β -D-glucosaminide α 1,6-fucosyltransferase VIII (FucT VIII) that adds Fuc in α 1,6 linkage to the Asn-linked GlcNAc in the *N*-glycan core. Many glycoproteins are known to carry *N*-glycans with a core α 1,6-Fuc, and it is therefore expected that the phenotype of these null mice is due to a complex interaction of many defective

glycoproteins. Indeed, the *fut8*^{−/−} mice showed severe growth retardation, and 70% died within 3 days after birth. The surviving mice suffered from emphysema-like changes in the lungs. These findings led to studies on proteins known to be associated with lung pathology. It was shown that the null mice exhibit a marked overexpression of matrix metalloproteinases (MMPs) and a down-regulation of extracellular matrix (ECM) proteins such as elastin, changes that are consistent with a deficiency in TGF- β 1 signaling (14–16). The authors established deficient TGF- β 1 signaling by carrying out TGF- β 1 binding assays and Smad2 phosphorylation analysis. The TGF- β 1 signaling deficiency was rescued by transfection of mouse *fut8*^{−/−} cells with the wild-type *fut8* gene.

Core fucosylation of TGF- β 1 receptor(s) is crucial for proper signaling in the lung.

Although this clever and exhaustive analysis proved that the fucosylation defect caused a decrease in TGF- β 1 signaling, it did not establish which fucosylated protein (or proteins) was responsible. The authors hypothesized that the TGF- β 1 receptor was the FucT VIII target protein responsible for the phenotype and proved it with an important experiment. Intraperitoneal injection of exogenous TGF- β 1 into 18-day-old *fut8*^{−/−} mice resulted in significant rescue of the emphysema-like phenotype and reduced MMP expression in the lung. The most likely explanation of this experiment is that the *fut8*^{−/−} TGF- β 1 receptor is only partially deactivated by lack of core Fuc and therefore excess TGF- β 1 can overcome the block. Although it is possible that a core fucosylated protein (or proteins) downstream of the receptor could be the FucT VIII target protein responsible for the *fut8*^{−/−} phenotype, this is unlikely because the downstream proteins are intracellular and not likely to be *N*-glycosylated.

I am not aware of previous articles on target glycoprotein-specific rescue of a glycosylation-deficient animal although an analogous example was recently reported in abstract form[†] and other examples may be in the literature. Mice lacking the two major polysialyltransferases (ST8Sia-II and ST8Sia-IV) involved in polysialic acid biosynthesis appear normal at birth but die precociously.[‡] The lethal phenotype was rescued by additional deletion of the gene encoding neural cell adhesion molecule (NCAM), indicating that polysialylation of NCAM has an essential function in controlling NCAM interactions during mouse development.

Both examples of rescue of a specific glycosyltransferase target involve special circumstances. This approach is clearly not generally applicable to the elucidation of specific mechanisms responsible for a phenotype. Many glycoproteins carry core α 1,6-fucosylated *N*-glycans and, as Wang *et al.* (13) discuss in their article in some detail, associations have been reported between *N*-glycan core α 1,6-fucosylation and various other functions. The *fut8*^{−/−} mouse can therefore be expected to show phenotypic changes because of the defective functions of glycoproteins other than the TGF- β 1 receptor. There are indeed abnormalities in the *fut8*^{−/−} mouse that are not rescued by exogenous TGF- β 1. However, the results reported by Wang *et al.* (13) have proved, at least to my satisfaction, that the core fucosylation of TGF- β 1 receptor(s) is crucial for proper function of TGF- β 1 signaling in the lung.

It is of interest to consider studies on other fucose-deficient mice and humans. Protein *O*-fucosyltransferase 1 (protein *O*-FucT) adds Fuc in α 1-*O*-glycosidic linkage to Ser and Thr residues of Notch and other proteins with EGF-like repeats. *O*-linked Fuc is essential for normal binding of Notch to its ligands.

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See companion article on page 15791.

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[†]Gerardy-Schahn, R., Weinhold, B., Rockle, I., Muhlenhoff, M., Seidenfaden, R., Marth, J. D. & Hildebrandt, H. (2005) *Glycoconjugate J.* 22, 188, abstr.

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Mouse embryos lacking protein *O*-FucT die at midgestation with severe defects in somitogenesis, vasculogenesis, cardiogenesis, and neurogenesis (17).

Fringe is an *N*-acetylglucosaminyltransferase that adds GlcNAc in β 1,3 linkage to Fuc- α 1-O-Ser/Thr on Notch and therefore cannot function in the absence of protein *O*-FucT. Fringe modifies the binding of Notch to its ligands. Mouse embryos with targeted disruption of lunatic fringe fail to form boundaries between individual somites and show other developmental abnormalities (18–20).

α 1,3-FucT IV and VII add Fuc in α 1,3 linkage to the GlcNAc of Gal β 1,4GlcNAc although the enzymes differ in their substrate specificities and sites of expression. FucT VII adds α 1,3-Fuc to P-selectin glycoprotein ligand 1 (PSGL-1), and FucT IV adds α 1,3-Fuc to E-selectin ligand 1 (ESL-1). Mice with null mutations in *fut4* and *fut7* (the genes encoding FucT IV and VII, respectively) (21–26) show loss of selectin-dependent leukocyte adhesion, leukocyte recruitment, and lymphocyte homing.

α 1,2-FucT1 and 2 (encoded by genes *fut1* and *fut2*, respectively) both add Fuc in α 1,2 linkage to the terminal Gal residue of Gal β 1,3GlcNAc, Gal β 1,4GlcNAc, and other β -galactosides (27). The human *FUT1* and *FUT2* genes correspond,

respectively, to the *H* and *Se* (secretor) blood group genes (27). Both *fut1*- and *fut2*-null mice develop normally and exhibit no gross phenotypic abnormalities (28).

The *FX* locus encodes GDP-4-keto,6-deoxy-Man epimerase/reductase, an enzyme in the *de novo* pathway of GDP-fucose synthesis. *FX*-null mice exhibit a virtually complete deficiency of cellular

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fucosylation and a variable frequency of intrauterine death (29). A severe human congenital disease, leukocyte adhesion deficiency type II (LAD-II), now called congenital disorder of glycosylation type IIc (CDG-IIc), is due to a defective GDP-Fuc transporter gene. CDG-IIc patients present with severe mental retardation, stunted growth, persistently elevated peripheral leukocytes, and re-

current bacterial peripheral infections. Surviving adult *FX*-null mice show neutrophilia, myeloproliferation, and absence of leukocyte selectin ligand expression reminiscent of CDG-IIc. Some patients with CDG-IIc respond to therapy with oral L-fucose (30, 31). It is therefore of interest that newborn and adult *FX*^{-/-} mice improve on a diet supplemented with fucose (29). The dietary fucose is converted to GDP-Fuc by an alternate salvage pathway independent of the *FX* locus.

Protein fucosylation is clearly implicated in the functions of the TGF- β 1 receptor, Notch, Fringe, and selectin ligands. The severe phenotypes of *FX*-null mice and humans with CDG-IIc show that there may be other proteins that require fucosylation for their functions. A similar situation exists for the glycosylation of proteins by other sugars. Powerful mass spectrometric methods are being applied by various international consortia to determine the glycan structures of as many glycoproteins as possible. The great challenge for glycobiology in the future is to determine the role of protein-bound glycans in the function of a specific protein for thousands of proteins. The article by Wang *et al.* (13) offers an approach that works under special circumstances. Other methods will have to be developed to meet this most difficult challenge.

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