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REVIEW

Fucose: biosynthesis and biological function in mammals

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Fucose is a deoxyhexose that is present in a wide variety of organisms. In mammals, fucose-containing glycans have important roles in blood transfusion reactions, selectin-mediated leukocyte-endothelial adhesion, host–microbe interactions, and numerous ontogenic events, including signaling events by the Notch receptor family. Alterations in the expression of fucosylated oligosaccharides have also been observed in several pathological processes, including cancer and atherosclerosis. Fucose deficiency is accompanied by a complex set of phenotypes both in humans with leukocyte adhesion deficiency type II (LAD II; also known as congenital disorder of glycosylation type IIc) and in a recently generated strain of mice with a conditional defect in fucosylated glycan expression. Fucosylated glycans are constructed by fucosyltransferases, which require the substrate GDP-fucose. Two pathways for the synthesis of GDP-fucose operate in mammalian cells, the GDP-mannose-dependent *de novo* pathway and the free fucose-dependent salvage pathway. In this review, we focus on the biological functions of mammalian fucosylated glycans and the biosynthetic processes leading to formation of the fucosylated glycan precursor GDP-fucose.

Key words: fucose/fucosyltransferase/Notch/selectins

Introduction

L-fucose (6-deoxy-L-galactose) is a monosaccharide that is a common component of many N- and O-linked glycans and glycolipids produced by mammalian cells. Two structural features distinguish fucose from other six-carbon sugars present in mammals. These include the lack of a hydroxyl group on the carbon at the 6-position (C-6) and the L-configuration. Fucose frequently exists as a terminal modification of glycan structures; however, recently glycosyltransferase activities capable of adding sugars directly to fucose have been identified (Moloney and Haltiwanger, 1999). Specific terminal glycan modifications, including fucosylation, can confer unique functional properties to oligosaccharides and are often regulated during ontogeny and cellular differentiation. Important roles for fucosylated glycans have been demonstrated in a variety of biological settings (Listinsky *et al*., 1998; Staudacher *et al*., 1999), several of which are reviewed shortly. However, because of the diversity of fucose-containing glycoconjugates and the difficulties inherent in studying the biological function of carbohydrates, it is likely that many additional functions for fucosylated glycans remain to be uncovered.

Fucosylated glycans are synthesized by fucosyltransferases (Table I). Thirteen fucosyltransferase genes have thus far been identified in the human genome. *FUT1* and *FUT2* are α(1,2)-fucosyltransferases responsible for synthesis of the H blood group antigen and related structures (Kelly *et al*., 1995; Larsen *et al*., 1990). *FUT3-FUT7* and *FUT9* encode fucosyltransferases that synthesize α(1,3)- and, in the case of *FUT3*, α(1,4)-fucosylated glycans, such as the Lewis^x and sialyl Lewis^x antigens (Kaneko *et al*., 1999; Natsuka and Lowe, 1994). *FUT8* is an α(1,6)-fucosyltransferase that directs addition of fucose to asparagine-linked GlcNAc moieties, a common feature of N-linked glycan core structures (Miyoshi *et al*., 1999). *POFUT1* encodes an O-fucosyltransferase that adds fucose directly to polypeptide chains (Wang *et al*., 2001). Finally, though not yet validated by functional studies, two additional putative α(1,3)-fucosyltransferase genes, *FUT10* and *FUT11*, and one additional putative O-fucosyltransferase gene, *O-FUT2*, have been identified in the human genome by comparison with fucosyltransferase sequences in the *Drosophila melanogaster* genome (Roos *et al*., 2002).

Fucosylated glycans: physiological and pathophysiological significance

Fucosylated glycans and the ABO blood group

The ABO blood group antigens are among the most well-known fucosylated glycans. The H transferase (*FUT1* gene product) is an α(1,2)-fucosyltransferase expressed in erythroid precursors that synthesizes a structure known as the H antigen by directing the addition of fucose to terminal galactose residues on oligosaccharide precursors decorating several glycoproteins and glycolipids (Figure 1A). In epithelial tissues and salivary glands, a second α(1,2)-fucosyltransferase known as the Secretor (Se) transferase (*FUT2* gene product) synthesizes the H antigen. In individuals of blood group A, B, or AB, the H antigen may be further

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Table I. Human fucosyltransferases

Common name(s)	HUGO name	Ref Seq/GenBank accession no	Representative major product(s)
H blood group α2fucosyltransferase	FUT1	NM_000148.1	**Fucα2**Galβ4GlcNAc-R
Secretor (Se) blood group α2fucosyltransferase	FUT2	NM_000511.1	**Fucα2**Galβ3GlcNAc-R
Fuc-TIII α3/4fucosyltransferase[a]	FUT3	NM_000149.1	Galβ4[**Fucα3**]GlcNAc-R
Lewis blood group fucosyltransferase			Siaα3Galβ4[**Fucα3**]GlcNAc-R
			Fucα2Galβ4[**Fucα3**]GlcNAc-R
			Galβ3[**Fucα4**]GlcNAc-R
			Siaα3Galβ3[**Fucα4**]GlcNAc-R
			Fucα2Galβ3[**Fucα4**]GlcNAc-R
Fuc-TIV α3fucosyltransferase[a]	FUT4	NM_002033.1	Galβ4[**Fucα3**]GlcNAcβ3Galβ4GlcNAc-R
ELAM-1 ligand fucosyl transferase (ELFT)			Galβ4]GlcNAcβ3Galβ4[**Fucα3**GlcNAc-R
			Galβ4[**Fucα3**]GlcNAcβ3Galβ4[**Fucα3**]GlcNAc-R
			SiααGalβ4GlcNAcβ3Galβ4[**Fucα3**]GlcNAc-R
			Siαα3Galβ4[**Fucα3**]GlcNAc-R
Fuc-TV α3fucosyltransferase[a]	FUT5	NM_002034.1	Galβ4[**Fucα3**]GlcNAc-R
			Siaα3Galβ4[**Fucα3**]GlcNAc-R
Fuc-TVI α3fucosyltransferase[a]	FUT6	NM_000150.1	Galβ4[**Fucα3**]GlcNAc-R
			Siaα3Galβ4[**Fucα3**]GlcNAc-R
Fuc-TVII α3fucosyltransferase	FUT7	NM_004479.1	Siaα3Galβ4[**Fucα3**]GlcNAc-R
Fuc-TVIII α6fucosyltransferase	FUT8	NM_004480.1	GNGNManβ4GlcNAcβ4[**Fucα6**]GlcNAc-Asn[b]
Fuc-TIX α3fucosyltransferase[a]	FUT9	NM_006581.1	Galβ4[**Fucα3**]GlcNAc-R
Fuc-TX putative α3fucosyltransferase[c]	FUT10	NM_032664.2	Unknown
Fuc-TXI putative α3fucosyltransferase[c]	FUT11	NM_173540.1	Unknown
Polypeptide O-fucosyltransferase	POFUT1	NM_015352.1	FucαSerine and FucαThreonine, within EGF repeats within a broad consensus site C(2)X(3–5)S/TC(3) (where X(3–5) are any 3–5 amino acid residues)
Putative polypeptide O-fucosyltransferase	POFUT2	NM_015227.1	Unknown

The fucose residue in bold type (Fuc) in each product corresponds to the fucose moiety added by the enzyme in the corresponding row.
[a]The α3fucosyltransferases exhibit a much more complex acceptor substrate specificity than is indicated by the representative major products illustrated in this table (see Nishihara *et al.*, 1999).
[b]GNGNManβ4GlcNAcβ4[**Fucα6**]GlcNAc-Asn is GlcNAcβ2Manα6[GlcNAcβ2Manα3]Manβ4GlcNAcβ4[**Fucα6**]GlcNAc-Asn.
[c]These proteins have not yet been demonstrated to be enzymatically active, although they share primary sequence similarity with proteins known to have fucosyltransferase activity.

modified by the *ABO* locus-encoded glycosyltransferase to form the A and B antigens; unmodified H antigen is expressed on the cell surface of type O individuals (Figure 1A) (Lowe, 1993). The A, B, and H antigens are highly immunogenic, and high titers of complement-fixing IgM against these molecules prevent successful red blood cell transfusions to incompatible individuals. The functional significance for ABO antigen expression on erthyrocytes has not been defined, but ABO-related structures may play a role in other systems.

Plasma levels of von Willebrand factor (VWF), an essential coagulation protein, vary widely between individuals, and genotype at the *ABO* and *Se* loci have been shown to be determinants of this variability. Because variation in VWF level contributes to disease severity in von Willebrand disease patients (Levy and Ginsburg, 2001), as well as predisposition toward clotting-related conditions, such as recurrent venous thromboembolism and ischemic heart disease in the general population (O'Donnell and Laffan, 2001), the medical importance of the ABO system may thus extend beyond blood typing issues. The molecular basis for modulation of VWF by fucosylated glycans has not been directly addressed experimentally, but a mechanism based on differential clearance of glycan-modified VWF by hepatic carbohydrate-recognition receptors has been proposed (Levy and Ginsburg, 2001).

Fucosylated glycans in host–microbe interactions

Fucosylated blood group antigens may also be important for host–microbe interactions. The gastric pathogen *Helicobacter pylori* is capable of attachment to the gastric epithelium via host expression of the Lewis[b] antigen, a structure containing α(1,2)- and α(1,4)-linked fucose that is synthesized by the concerted action of the Se and Lewis (*FUT3*) fucosyltransferases (Figure 1B). This Lewis[b]-determined interaction may

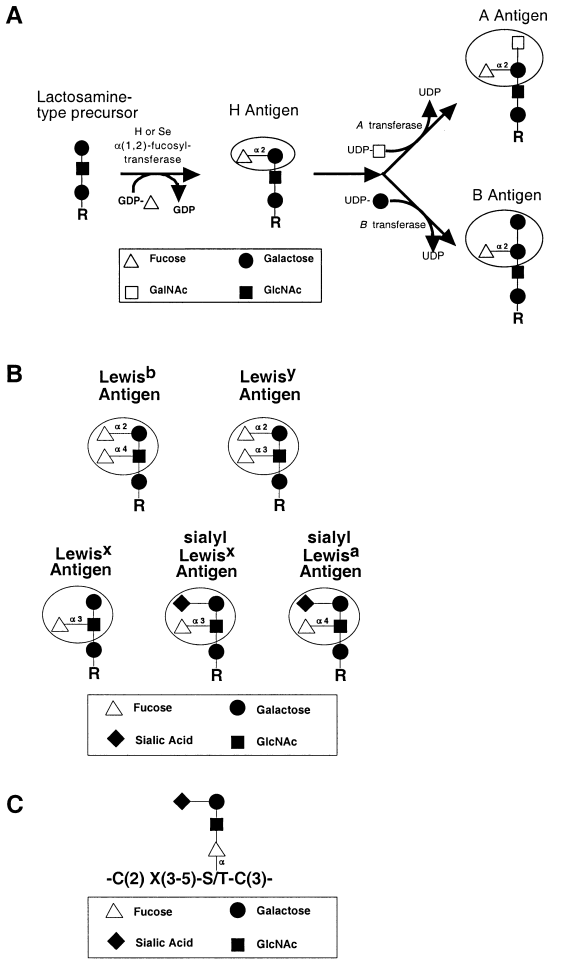

Fig. 1. Structures of common fucosylated glycans. (**A**) Synthesis of ABO blood group antigens. The H and Se transferases are a pair of α(1,2)-fucosyltransferases that synthesize the H antigen in a variety of tissues. The *ABO* locus encodes a glycosyltransferase that further modifies the H antigen. The *A* allele at the *ABO* locus encodes an N-acteylgalactosaminyltransferase. The *B* allele encodes a galactosyltransferase that differs from the A transferase at four amino acid positions. The *O* allele at the *ABO* locus encodes a truncated, enzymatically inactive protein. (**B**) Lewis-related antigens. Circles indicate the immunodominant portion of each antigen. (**C**) A representative O-linked fucose glycan. Fucose modifies serines or threonine within the broad consensus site shown here, and in Table I. R indicates glycolipid and N- and O-linked glycoprotein precursors.

contribute to development of *H. pylori*–mediated peptic ulcer disease because masking of Lewis^b with terminal GalNAc by the A transferase abolishes bacterial binding and correlates with the decreased ulcer incidence observed in A and B blood group individuals (Hooper and Gordon, 2001). Moreover, overexpression of the Lewis transferase in the gastric pit cells of mice, an organism that normally does not produce Lewis^b, leads to Lewis^b expression and increased severity of gastritis following *H. pylori* inoculation (Guruge *et al.*, 1998). In addition to Lewis antigen expression by the host, *H. pylori* cells also produce Lewis-related structures, such as Lewis^x, Lewis^y, and Lewis^b (Figure 1B), an example of molecular mimicry (Appelmelk *et al.*, 1997). Expression of these fucosylated glycans by *H. pylori* increases at acid pH (Moran *et al.*, 2002), coincident with up-regulation of a fucose biosynthetic gene (McGowan *et al.*, 1998), and may induce autoimmune-mediated damage to the gastric epithelium, leading to chronic atrophic gastritis in a subset of *H. pylori*–infected humans (Hooper and Gordon, 2001).

In addition to expression by some gastric cell lineages, glycans containing α(1,2)-linked fucose are also abundantly expressed by intestinal epithelium of adult mammals (Torres-Pinedo and Mahmood, 1984). During the weaning transition in rats, as the developing animal switches to solid food, the intestine undergoes a profound transformation that includes increased fucose biosynthetic capacity (Ruggiero-Lopez *et al.*, 1991), up-regulation of fucosyltransferase activity (Biol *et al.*, 1987), and increased fucosylated glycan expression. The shift toward fucosylation in the adult intestine can be accelerated by administration of insulin or prevented by prolonged suckling (Biol *et al.*, 1998), indicating that specific signals control this developmental program. Experiments with germ-free mice have demonstrated that maintenance of α(1,2)-fucosyltransferase mRNA and α(1,2)-fucosylated glycan expression after weaning depends on the presence of normal microflora, and colonization by a single component of the flora, *Bacteroides thetaiotamicron*, which restores fucosyltransferase and fucosylated glycan expression (Bry *et al.*, 1996). Moreover, an exquisite system for controlling the expression of fucose catabolism genes by sensing fucose availability has been described in *B. thetaiotamicron*. Indirect genetic evidence suggests that the fucose-sensing protein in *B. thetaiotamicron* is also capable of inducing the host intestine to increase expression of fucosylated glycans and thereby increase bacterial fucose supply (Hooper *et al.*, 1999). These observations imply that α(1,2)-fucosylated glycan expression contributes to establishment of the indigenous microbial community in the developing gut by providing a favorable ecological niche for specific nonharmful commensal organisms capable of utilizing fucose as a carbon source (Hooper *et al.*, 2000).

Contributions of fucosylated glycans to selectin-dependent leukocyte adhesion

One of the best-studied functions of fucose is its role as an essential component of the carbohydrate ligands for the selectin family of cell adhesion receptors (Kansas, 1996; Vestweber and Blanks, 1999). E-, P-, and L-selectin are C-type lectin proteins expressed by platelets (P-selectin), endothelial cells (E- and P-selectin), and leukocytes (L-selectin). Selectins bind to oligosaccharides, decorating specific cell surface and secreted proteins expressed by leukocytes (E- and P-selectin ligands) and high endothelial venules (L-selectin ligands). Interaction between selectins and their ligands enable the rolling of leukocytes on the endothelium, the required first step in leukocyte extravasation (Springer, 1994). The carbohydrate selectin ligands are fucosylated structures related to the sialyl Lewis^x structure (Figure 1B). Two α(1,3) fucosyltransferases, Fuc-TVII (*FUT7* gene product) and Fuc-TIV (*FUT4* gene product), are expressed in leukocytes and endothelial cells and catalyze the final reaction in selectin ligand biosynthesis, the addition of fucose to sialylated precursors (Lowe, 1997;

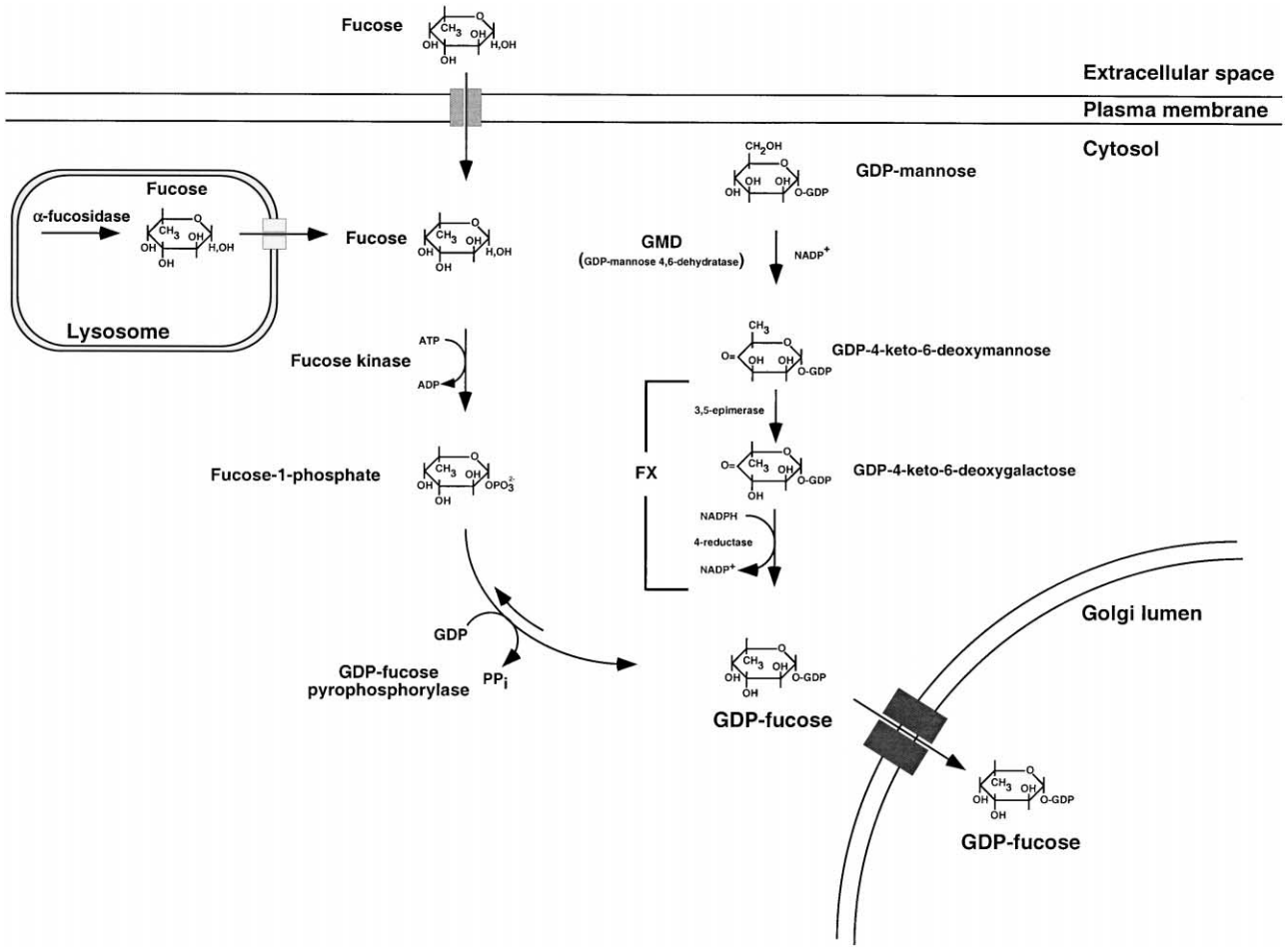

Fig. 2. Biosynthesis of GDP-fucose. In mammalian cells, GDP-fucose is synthesized by two distinct pathways. The *de novo* pathway is characterized by conversion of GDP-mannose to GDP-4-keto-6-deoxymannose by GMD. This keto intermediate is then converted to GDP-fucose by an epimerase/reductase known as the FX protein. An alternative salvage pathway can yield GDP-fucose derived directly from fucose. The salvage pathway utilizes fucose that is transported into the cytosol from an extracellular origin or fucose that is liberated from catabolism of fucosylated glycans in the lysosome and then transported into the cytosol. The salvage pathway is enabled by fucose kinase and GDP-fucose pyrophosphorylase, with fucose-1-phosphate is the metabolic intermediate. GDP-fucose synthesized by these pathways is then transported into the lumen of the Golgi apparatus where it becomes available to the catalytic domains of fucosyltransferases that also localize to this membrane-delimited compartment within the secretory pathway.

Natsuka and Lowe, 1994; Smith *et al.*, 1996). Gene ablation experiments in mice have established that Fuc-TVII is essential for proper recruitment of neutrophils and T cells to inflammatory sites and lymphocyte trafficking to secondary lymphoid organs (Maly *et al.*, 1996; Smithson *et al.*, 2001). In contrast, although neutrophils in Fuc-TIV–/– mice have been observed to roll on the dermal microvasculature at a higher velocity than wild-type neutrophils (Weninger *et al.*, 2000), the contribution of Fuc-TIV to neutrophil and lymphocyte recruitment is much smaller than the contribution of Fuc-TVII and has thus far been apparent only in the context of deficiency for both Fuc-TIV and Fuc-TVII (Homeister *et al.*, 2001; Smithson *et al.*, 2001).

Fucosylated glycans in development

There is evidence for involvement of fucosylated glycans in ontogenic events. The Lewis^x epitope, an α(1,3)-fucosylated glycan also known as the stage-specific embryonic antigen-1 and CD15, is expressed during early embryogenesis (Solter and Knowles, 1978). Exposure of preimplantation mouse embryos at the morula stage to oligosaccharides containing multivalent Lewis^x structures causes decompaction, implying that Lewis^x promotes cell–cell adhesion in early embryos (Bird and Kimber, 1984; Fenderson *et al.*, 1984). Carbohydrate–carbohydrate interactions, that is, Lewis^x interacting with itself, are thought to form the molecular basis for Lewis^x-mediated embryo compaction (Eggens *et al.*, 1989). Lewis^x epitopes are also present in multiple areas of the embryonic brain (Ashwell and Mai, 1997a,b,c,d), controlled by expression of Fuc-TIX (*FUT9* gene product), which is in turn controlled by expression of Pax6, a transcription factor important for neural development (Shimoda *et al.*, 2002). The biological significance of Lewis^x in the developing brain is not known, but its dynamic expression pattern at different embryonic stages has led many to infer important functions during neurogenesis (Kudo *et al.*, 1998).

O-linked fucosylation of EGF-like domains and signal transduction

In contrast to its role as a terminal modification of oligosaccharides, fucose may also be found in direct linkage to hydroxyl groups of serine and threonine residues. This glycosylation event, known as O-fucosylation, is carried out by one or more Golgi-resident O-fucosyltransferases (Wang *et al*., 2001; Wang and Spellman, 1998) that act on specific consensus sequences in epidermal growth factor (EGF)-like modules of cell surface and secreted proteins, including several blood coagulation factors (Harris and Spellman, 1993; Moloney and Haltiwanger, 1999). O-fucose residues do not appear to affect tertiary protein structure because O-fucosylation of an EGF domain from Factor VII does not alter solution structure or binding affinity for tissue factor (Kao *et al*., 1999). However, O-fucosylation can have important functional consequences. Defucosylation of the EGF domain from urokinase-type plasminogen activator abolishes its mitogenic activity despite having no effect on binding to the cell surface (Rabbani *et al*., 1992), suggesting that O-fucose can qualitatively affect ligand–receptor interactions necessary for productive signal transduction events. Supporting this notion, mutation of an O-fucosylation site in the EGF domain of human Cripto-1, a glycosylphosphatidylinositol-anchored protein critical for early embryogenesis (Ding *et al*., 1998; Minchiotti *et al*., 2000), reduces its ability to facilitate signaling by the tumor growth factor β family member Nodal (Schiffer *et al*., 2001).

O-fucose residues are also present on EGF domains of the mammalian Notch receptors (Moloney *et al*., 2000b), a family of transmembrane signaling proteins with important roles in cell fate determination during somite formation, neurogenesis, angiogenesis, and lymphoid development (Artavanis-Tsakonas *et al*., 1999). Ligand-induced Notch signaling events are impaired in a fucose-deficient cell line but can be restored by correction of the fucosylation defect, implying that O-fucosylation of Notch affects its interaction with and/or its response to ligands (Chen *et al*., 2001; Moloney *et al*., 2000a). In *Drosophila*, O-linked fucose is apparently essential to Notch signaling events and corresponding cell fate determination (Okajima and Irvine, 2002). Notch signaling is modulated in a number of contexts by expression of *Fringe* genes (Panin and Irvine, 1998), which have recently been shown to encode glycosyltransferases that direct addition of GlcNAc to O-fucose residues on Notch (Bruckner *et al*., 2000; Moloney *et al*., 2000a). Fringe-mediated extension of O-fucose differentially impacts Notch activity in response to ligands, demonstrating that an additional function for O-fucose on Notch is to serve as the required substrate for Fringe (Bruckner *et al*., 2000; Hicks *et al*., 2000; Moloney *et al*., 2000a; Shimizu *et al*., 2001). Further, O-fucosylation of Notch and/or other yet-to-be-discovered Fringe substrates may be essential to normal mammalian development because targeted mutation of one of the *Fringe* genes in mice causes severe defects in somite formation and early demise (Evrard *et al*., 1998; Zhang and Gridley, 1998).

Recent evidence indicates that O-fucosylation is not limited to EGF domains, as glucose-extended fucose modifications (Glc-Fuc-*O*-Ser/Thr) have been demonstrated in three thrombospondin type 1 repeats of thrombospondin-1 (Hofsteenge *et al*., 2001). Functional investigation of O-fucosylation on thrombospondin type 1 repeats, however, has not yet been reported. It remains to be determined if additional protein modules are also modified by O-fucosylation.

Fucosylated glycans in human disease

Fucosylated glycans have been implicated in the pathogenesis of several human diseases. Two prominent examples of altered glycosylation in cancer involve fucose-containing oligosaccharides. First, expression of A and B blood group antigens is lost in many tumors with concomitant increases in H and Lewis^{y} expression, changes that correlate with poor clinical prognosis (Kim and Varki, 1997; Lee *et al*., 1991; Miyake *et al*., 1992; Orntoft and Vestergaard, 1999). Second, up-regulation of sialyl Lewis^{x} and sialyl Lewis^{a} (Figure 1B) has been demonstrated in numerous cancers, and these increases are also associated with advanced tumor grade and poor prognosis. Sialyl Lewis^{x} and sialyl Lewis^{a} serve as ligands for the selectin molecules and thus may aid in hematogenous metastasis by direct binding of cancer cells to E- and P-selectin expressed by the endothelium (Kannagi, 1997; Kim and Varki, 1997). Other possible mechanisms for sialyl Lewis$^{x/a}$-mediated metastasis include formation of cellular thromboemboli via interaction with platelet-borne P-selectin (Borsig *et al*., 2001) and blockade of leukocytic infiltration into tumors by secretion of sialyl Lewis$^{x/a}$-containing inhibitors of leukocyte-endothelial adhesion (Kim and Varki, 1997). Moreover, increased α(1,6)-fucosylation of α-fetoprotein is observed in hepatocellular carcinoma patients and can be used clinically as a marker for distinguishing hepatocellular carcinoma from chronic liver disease (Miyoshi *et al*., 1999).

Increased expression of fucosylated glycans has also been reported on serum immunoglobins in both juvenile and adult rheumatoid arthritis patients (Flogel *et al*., 1998; Gornik *et al*., 1999). It is not known if such changes are important to the pathogenesis of inflammatory arthritis or if they represent a secondary consequence due to up-regulation of the fucosylation machinery in the context of autoimmunity. Similarly, fucosylation of mucins has been observed to be increased in cystic fibrosis, with a concomitant decrease in sialylation (Scanlan and Glick, 1999). In a murine model of this disease, increased fucosylation of small bowel mucin correlates with substantially increased expression of the murine Fut2 α(1,2)-fucosyltransferase mRNA (Thomsson *et al*., 2002). It remains to be determined if there are causal relationships between these observations and aberrant microbial colonization that characterize the pathogenesis of cystic fibrosis. Last, as a result of their role in leukocyte recruitment, selectin–selectin ligand interactions contribute to the development of numerous pathological processes, including atherosclerosis, reperfusion injury following ischemic events, inflammatory skin diseases, and asthma (Varki, 1999).

Mammalian cell lines that lack cell surface fucosylated glycan expression are viable with no apparent defect in cell division (Reitman *et al*., 1980; Ripka *et al*., 1986). This is perhaps not surprising because many of the functions of fucosylated glycans depend on cell–cell interactions,

developmental processes, or host–microbe interactions. However, mammals exhibiting a global deficiency in fucosylated glycans have severe defects in multiple systems. Sharp reductions in cell surface fucosylated glycans are observed in humans with leukocyte adhesion deficiency type II (LAD II; also known as congenital disorder of glycosylation IIc) (Becker and Lowe, 1999). These patients suffer recurrent infections consequent to defective selectin ligand biosynthesis. In addition, mental retardation and skeletal abnormalities are also prominent features in LAD II, but it is not known if these conditions result from defects in fucose-dependent processes, such as O-fucosylation of Notch receptors or Lewis^x interactions in the embryonic brain. The molecular basis of LAD II is discussed in more detail (see *Salvage pathway*). Mice engineered to have a conditional impairment in fucosylated glycan expression also exhibit a pleiotropic phenotype that includes altered myeloid development, diarrhea with inflammatory bowel disease-like histology, and infertility (Smith *et al*., 2002). Because fucosylated glycan expression can be reversibly and rapidly controlled in these mice, this knockout mouse strain should be an important tool for elucidating the many previously unrecognized and unexplored functions for fucose-containing glycoconjugates. The observation that fucose deficiency in animals causes a large number of phenotypic consequences underscores the crucial role of fucosylated glycans to many physiological and developmental processes.

Fucose metabolism

All fucosyltransferases utilize a nucleotide-activated form of fucose, GDP-fucose, as a fucose donor in the construction of fucosylated oligosaccharides. Two pathways have been described for synthesis of GDP-fucose in the cytosol of essentially all mammalian cells. These are termed the *de novo* pathway and the salvage pathway (Figure 2) (Tonetti *et al*., 1998). The *de novo* pathway transforms GDP-mannose to GDP-fucose via three enzymatic reactions carried out by two proteins, GDP-mannose 4,6-dehydratase (GMD) and a second enzyme, GDP-keto-6-deoxymannose 3,5-epimerase, 4-reductase, also known as the FX protein (Tonetti *et al*., 1996). The salvage pathway synthesizes GDP-fucose from free fucose derived from extracellular or lysosomal sources. Quantitative studies of fucose metabolism in HeLa cells indicate that greater than 90% of GDP-fucose is derived from the *de novo* pathway, even in cells fed radiolabeled fucose (Yurchenco and Atkinson, 1975, 1977). Nevertheless, as will be discussed, the existence of the salvage GDP-fucose biosynthetic pathway has proven useful for correction of fucosylation defects in LAD II patients, FX–/– mice, and mutant cell lines and thus has considerable importance for the study of fucosylation processes in mammals. Both pathways are discussed in detail in the following sections.

De novo *pathway*

The *de novo* pathway for GDP-fucose biosynthesis was first proposed over 40 years ago by Victor Ginsburg, whose pioneering work demonstrated that GDP-mannose was converted to GDP-fucose via a keto-containing intermediate, GDP-4-keto-6-deoxymannose (Ginsburg, 1960, 1961a,b). The first reaction in the *de novo* pathway is carried out by GMD, which converts GDP-mannose to GDP-4-keto-6-deoxymannose by catalyzing the oxidation of the hydroxyl group at C-4 of the mannose ring coupled with reduction of the hydroxyl at C-6 (Figure 2). GMD requires a stably bound nicotinamide adenine dinucleotide phosphate$^+$ (NADP) cofactor, which serves as a shuttle for transfer of electrons from C-4 to C-6 (Chang *et al*., 1985; Oths *et al*., 1990; Somoza *et al*., 2000; Sullivan *et al*., 1998; Yamamoto *et al*., 1993).

GMD is conserved throughout evolution; nucleotide sequences for GMD from over 20 bacterial species, plants (*Arabidopsis*), invertebrates (*C. elegans*, *D. melanogaster*), and mammals (human, mouse expressed sequence tag) have been deposited in GenBank (Becker, unpublished data; Lamrabet *et al*., 1999; Ohyama *et al*., 1998; Roos *et al*., 2002; Somoza *et al*., 2000; Stevenson *et al*., 1996; Sullivan *et al*., 1998). GMD transcripts are ubiquitously expressed in most or all human tissues, although the transcript levels vary considerably between tissues (Sullivan *et al*., 1998). The crystal structure of *Escherichia coli* GMD has been solved (Somoza *et al*., 2000), demonstrating that GMD is a member of the short chain dehydrogenase/reductase (SDR) family (Jornvall *et al*., 1995). GMD is a homodimeric protein with each monomer consisting of two domains, an NADP$^+$ binding N-terminal domain and a nucleotide sugar binding C-terminal domain (Somoza *et al*., 2000). GDP-fucose is a potent competitive inhibitor of GMD (Albermann *et al*., 2000; Bisso *et al*., 1999; Broschat *et al*., 1985; Kornfeld and Ginsburg, 1966; Somoza *et al*., 2000; Sturla *et al*., 1997; Sullivan *et al*., 1998), thus demonstrating a classic example of feedback inhibition by the final product of the pathway.

GDP-4-keto-6-deoxymannose produced by GMD is then converted to GDP-fucose by a dual functional epimerase-reductase enzyme known as the FX protein (Chang *et al*., 1988; Tonetti *et al*., 1996). In the first reaction carried out by the FX protein, the hydroxyl group at C-3 and the methyl group at C-5 of the mannose ring are epimerized to yield GDP-4-keto-6-deoxygalactose. The 4-reductase activity of FX protein then catalyzes a hydride transfer from the required Nicotinamide adenine dinucleotide phosphate, reduced form, (NADPH) cofactor to the keto group at C-4, yielding GDP-fucose and NADP$^+$ (Figure 2) (Menon *et al*., 1999).

The FX protein was first isolated and characterized in the mid-1970s by Antonio De Flora and colleagues who, while purifying glucose-6-phosphate dehydrogenase from human erthyrocytes, observed the presence of an additional, contaminating NADP(H)-binding protein. As their initial studies failed to determine the identity of the copurified protein, they coined the moniker FX to indicate its unknown function (De Flora *et al*., 1975, 1977b; Morelli *et al*., 1977; Morelli and De Flora, 1977). Twenty years after its initial identification, De Flora and colleagues determined by amino acid sequencing that the FX protein is the human homolog of a previously characterized mouse protein known as tissue-specific transplantation antigen P35B (Camardella *et al*., 1995). A dominantly acting point mutation in the gene encoding P35B causes major

histocompatibility complex (MHC)-mediated immune rejection and loss of tumorgenicity in a mouse mastocytoma clone (Szikora *et al*., 1990). Although it is not known if the P35B mutation affects FX enzymatic function in the mastocytoma clone, the observation that the P35B amino acid substitution generates an MHC-binding neoantigenic peptide (Szikora *et al*., 1993) implies that the role of the FX protein as a transplantation antigen is unrelated to its function in GDP-fucose biosynthesis. However, examination of the P35B (i.e., FX) cDNA sequence disclosed homology to bacterial genes encoding enzymes proposed to mediate nucleotide sugar metabolism, leading to the discovery that the FX protein represents the mammalian GDP-fucose synthetase (Tonetti *et al*., 1996). Comparison between FX cDNA sequences disclosed a remarkable evolutionary conservation with 93% sequence identity (98% similarity) between human and mouse, and 50% identity of a 670 nucleotide region between human and *E. coli* (Tonetti *et al*., 1996). The crystal structure of the *E. coli* homolog of the FX protein has been determined, revealing that the FX protein is also a member of the SDR family with a structure similar to GMD, including homodimerization and a two-domain structure of the monomer subunits analogous to the domains of GMD (Rizzi *et al*., 1998; Rosano *et al*., 2000; Somers *et al*., 1998).

Biosynthesis of GDP-fucose from GDP-mannose can be reconstituted *in vitro*, both with cytosolic extracts and recombinant enzymes. Multiple laboratories working with many different tissues and species have observed that the activity of GMD in cellular extracts can be measured independently from FX activity by omitting the FX proteins required NADPH cofactor from the reaction mixture (Bonin *et al*., 1997; Ginsburg, 1961b; Liao and Barber, 1971; Reitman *et al*., 1980; Ripka *et al*., 1986). In the absence of exogenous NADPH, both the epimerase and reductase functions of the FX protein are apparently inactive, leading to accumulation of the product of GMD, GDP-4-keto-6-deoxymannose.

Cytosolic assays performed in the presence of NADPH measure the concerted action of GMD and FX. The one published exception to this paradigm was observed in a study of Nereid seaworm lysates. In that study, significant production of GDP-4-keto-6-deoxygalactose, the product of the epimerase activity of the FX protein, was observed in the absence of NADPH (Bulet *et al*., 1984). Molecular cloning of the cDNAs encoding GMD and FX allowed for reconstitution of the *de novo* pathway with recombinant, bacterially expressed GMD and FX proteins (Sullivan *et al*., 1998). Reactions with recombinant GMD and millimolar quantities of GDP-mannose proceed to completion. Subsequent addition of FX protein and NADPH yields stoichiometric quantities of GDP-fucose (Albermann *et al*., 2000; Sullivan *et al*., 1998). "One-pot" synthesis of GDP-fucose from GDP-mannose is not possible, however, because of feedback inhibition of GMD by GDP-fucose (Albermann *et al*., 2000). Experiments with the *E. coli* homolog of FX protein reveal that the epimerase activity can be active in the absence of NADPH, at least for recombinant protein in the presence of high substrate concentrations (Menon *et al*., 1999).

There is evidence that the *de novo* GDP-fucose biosynthetic pathway is subject to regulatory control in a variety of biological contexts. For example, GMD and FX activities increase during the nutrional transition associated with weaning in the rat intestine (Ruggiero-Lopez *et al*., 1991), in parallel with increased intestinal fucosyltransferase activities, and fucosylated glycan expression (Chu and Walker, 1986; Torres-Pinedo and Mahmood, 1984). These observations imply the existence of mechanisms that coordinately control the elaboration of fucosylated glycans. Dynamic control of GMD activity has also been observed in a species of Nereid seaworms, where oocyte maturation is accompanied by increased GMD activity (Bulet *et al*., 1984) and a concomitant increase in the content of protein-bound fucose. Regulated increases in the expression of FX mRNA and/or FX protein have been observed in glucose-6-phosphate dehydrogenase deficiency in human erthyrocytes (De Flora *et al*., 1977a), in association with acid pH stress in *H. pylori* (McGowan *et al*., 1998), following antibody-mediated stimulation of human squamous cell carcinoma cell lines (Eshel *et al*., 2000), and after polyclonal activation of human lymphocytes (Eshel *et al*., 2001).

Salvage pathway

The existence of an alternative salvage pathway for GDP-fucose biosynthesis was first inferred from studies in which radiolabeled fucose was administered to rats or provided in the culture medium of HeLa cells (Coffey *et al*., 1964; Kaufman and Ginsburg, 1968). These experiments demonstrated that fucose is directly incorporated into glycoproteins with little or no conversion to other sugars, thus indicating that fucose can be directly "activated" to GDP-fucose by means of a pathway independent of GDP-mannose. Free fucose derived from dietary sources or, in the case of cultured cells, from the culture medium, is transported across the plasma membrane into the cytosol. Relatively little is known about the cell surface fucose transport system, but it appears to operate by facilitated diffusion and to be specific for fucose (Wiese *et al*., 1994). In this context, there is evidence that in rats, fucose is a competitive inhibitor of a plasma membrane sodium-dependent myo-inositol transport system and that very high levels of dietery fucose can deplete cellular myo-inositol pools (Yorek *et al*., 1993). However, the molecular relationship between plasma membrane transporter(s) for myo-inositol and fucose transporters is not yet defined. Free fucose that supplies the salvage pathway may also derive from lysosomal catabolism of glycoproteins and glycolipids by one or more fucosidase activities (Johnson and Alhadeff, 1991; Michalski and Klein, 1999). Fucose liberated in the lysosomal compartment can be transported across the lysosomal membrane into the cytosol by a relatively uncharacterized transport system that appears to allow efflux of multiple neutral sugars by facilitated diffusion (Jonas *et al*., 1990).

Free fucose in the cytosol is converted to GDP-fucose by a two-reaction pathway. The first step is carried out by the fucose kinase enzyme, which synthesizes fucose-1-phosphate from fucose with consumption of ATP and liberation of ADP (Ishihara *et al*., 1968; Park *et al*., 1998; Richards *et al*., 1978). GDP-fucose pyrophosphorylase (GFPP) then catalyzes the reversible condensation of fucose-1-phosphate with GTP to form GDP-fucose (Ishihara and Heath, 1968;

Pastuszak *et al*., 1998). Fucose kinase activity has been detected in multiple tissues (Park *et al*., 1998), and GFPP transcripts and/or biochemical activities have been detected in all tissues tested (except for one immortalized cell line) (Pastuszak *et al*., 1998), indicating that the fucose salvage pathway is a common, perhaps even universal, feature of mammalian cell types. In analogy to feedback inhibition of GMD, fucose kinase is potently inhibited by GDP-fucose (Park *et al*., 1998; Richards *et al*., 1978). There is also evidence that the activity of fucose kinase is regulated in response to assorted stimuli, including stimulation of the brain by dopaminergic pathways and exposure of aortic endothelial cells to nicotine (Hocher *et al*., 1993; Jork *et al*., 1984; Ricken *et al*., 1990).

GDP-fucose produced in the cytosol by either biosynthetic pathway must then enter the Golgi apparatus where it serves as a substrate in the synthesis of fucosylated glycoconjugates by fucosyltransferases. Golgi uptake of GDP-fucose is achieved by a specific transport protein and is coupled with obligatory antiport of GMP into the cytosol (Cacan *et al*., 1984; Capasso and Hirschberg, 1984; Hirschberg *et al*., 1998; Puglielli and Hirschberg, 1999; Sommers and Hirschberg, 1982). Golgi preparations from LAD II cells exhibit reduced GDP-fucose transport activity (Lubke *et al*., 1999; Sturla *et al*., 2001). This observation recently led to the cloning of a cDNA encoding the Golgi GDP-fucose transporter by complementation of LAD II fibroblasts and the identification of mutations in the corresponding gene in LAD II patients (Lubke *et al*., 2001; Luhn *et al*., 2001).

The available evidence indicates that the salvage pathway makes a minor contribution to cellular GDP-fucose pools under normal conditions. Although the amount of fucose in a typical modern human diet has not been well studied, fucose is present in several nonstarch polysaccharides and is a component of glycoproteins and glycolipids from nearly every species. Thus, depending on dietary composition and bioavailability of the fucose in plant or animal glycans, humans and other mammals are likely to ingest variable amounts of fucose. These subjects have not yet been carefully studied. In addition, intestinal microflora synthesize fucosylated glycans that could be catabolized to generate free fucose with subsequent uptake by the colon.

In limited clinical experience with LAD II patients, fucosylated glycan expression is severely impaired in multiple cell types in the absence of fucose supplementation (Etzioni *et al*., 1992; Frydman *et al*., 1992; Marquardt *et al*., 1999a,b). These observations demonstrate that at least in a handful of children fed normal diets, flux through the salvage pathway does not generate cytosolic GDP-fucose concentrations sufficient to overcome the relatively modest defect in transport of GDP-fucose into the Golgi that is characteristic of LAD II cells. Provision of oral fucose, however, restored fucosylation to one LAD II patient (Marquardt *et al*., 1999b), indicating that the salvage pathway maintains the capacity to generate GDP-fucose concentrations sufficient to overcome defective GDP-fucose import in this disease if supplied with supraphysiological concentrations of fucose. In support of this conclusion, glycoconjugates are undetectable in multiple adult tissues in mice with a targeted mutation of the *FX* locus unless exogenous fucose is supplied in the chow or water (Smith *et al*., 2002). These results in fucosylation-deficient humans and mice recapitulate findings with cultured mammalian cell lines with defects in GMD (Reitman *et al*., 1980; Ripka *et al*., 1986) or the FX protein (Becker *et al*., data not published). Disabling the *de novo* pathway in these cell lines abolishes cell surface fucosylated glycan expression, implying that the amount of fucose and fucosylated glycoconjugates in standard fetal bovine serum–containing cell culture medium is inadequate to correct fucosylation via supply of the salvage pathway. This observation is consistent with the relative paucity of fucosylation of plasma glycoproteins and contrasts with the relatively greater abundance of galactose and N-acetylgalactosamine on such glycoproteins. Such glycoproteins, when endocytosed and catabolized in the lysosome, can restore defective O-glycosylation characteristic of ldlD Chinese hamster ovary cells, a UDP-galactose/UDP-N-acetylgalactosamine 4-epimerase-deficient cell mutant in which UDP-galactose and UDP-N-acetylgalactosamine synthesis is disabled unless circumvented by free galactose (Kingsley *et al*., 1986).

Other pathways of fucose metabolism in mammalian cells?

Additional pathways for GDP-fucose biosynthesis have not been described in mammalian cells. Bacteria are capable of growth on fucose as the sole carbon and energy source by means of a fucose-inducible operon encoding fucose catabolic enzymes. The steps in this pathway include entry of free fucose into the bacterial cell through a permease protein, isomerization of fucose (an aldose) to form fuculose (a ketose; C 2 is a keto group, whereas C 1 is no longer an alcohol), phosphorylation of fuculose to form fuculose-1-phosphate, and an aldolase reaction to form lactaldehyde and dihydroxyacetone phosphate from fuculose-1-phosphate (Chen *et al*., 1987; Hooper *et al*., 1999; Zhu and Lin, 1988). Early investigations of bacterial fucose metabolism during the 1950s proposed the possibility that fucose might be synthesized from two three-carbon units, either by a pathway operating in reverse from the fucose catabolic pathway or by variations on this theme (Huang and Miller, 1958a,b). However, evidence supporting this proposed fucose synthetic pathway has never been reported.

Fucose catabolism by bacteria is well described, varies according to the species, and can be robust enough to allow some bacteria to use fucose as a carbon source (Dahms and Anderson, 1972; Hacking and Lin, 1976). These observations imply that free fucose in the diet, or released in the digestive tract from ingested fucose-containing foods, may be diverted to catabolic pathways before absorption by the digestive tract. The degree to which intestinal bacteria contribute to oral fucose disposition is not understood. In contrast, a catabolic pathway for utilization of free fucose as an energy source has not been identified in mammalian cells. However, in a study involving intravenous administration of [1-^{14}C]fucose to human patients, it was reported that 39% of the injected radioactivity was excreted as $^{14}CO_2$ (Segal and Topper, 1960), suggesting that fucose could be metabolized to smaller metabolic units. Subsequent work

in rats corroborated this finding, but the proportion of administered fucose that was metabolized in this manner was much smaller, with only 1.6% of the intraperitoneally injected [1-^{14}C] fucose appearing as $^{14}CO_2$ (Bekesi and Winzler, 1967).

In contrast, injection of milligram quantities of free fucose to mice did not lead to formation of glycogen (Shull and Miller, 1960), implying that fucose is not broken down into three-carbon glycogenic intermediates and thus diminishing the likelihood that fucose is catabolized in mammals by a pathway analogous to the bacterial catabolism. These observations contrast with biochemical studies of fucose metabolism in pigs (Nwokoro and Schachter, 1975a,b; Chan *et al*., 1979). This *in vitro* work identifies a catabolic pathway in extracts of porcine hepatocytes that can convert 1 mol of fucose to 2 mol of L-lactate (Chan *et al*., 1979). The three assayable enzyme activities in this pathway are found in the liver and kidney and to a lesser extent in other porcine tissues, but only one of the four is found in rat hepatocytes (Chan *et al*., 1979). It is not yet known if this catabolic pathway operates in other species, nor is it yet clear if it contributes significantly to fucose metabolism *in vivo*.

Although alternative pathways for fucose metabolism cannot be excluded, the fact that *de novo* pathway mutant mice and cell lines are crippled in their ability to express fucosylated glycans suggests that the contribution of any additional fucose synthetic pathways to the cellular GDP-fucose pool must be very small or nonexistent in most cell types of adult mammals. It remains to be determined if additional GDP-fucose biosynthetic pathways are active in specific circumstances during development or differentiation or in specialized mammalian cell types.

Abbreviations

EGF, epidermal growth factor; FX, GDP-keto-6-deoxy-mannose 3,5-epimerase, 4-reductase; GFPP, GDP-fucose pyrophosphorylase; GMD, GDP-mannose 4,6-dehydratase; LAD, leukocyte adhesion deficiency; MHC, major histocompatibility complex; NADP, Nicotinamide adenine dinucleotide phosphate; NADPH, Nicotinamide adenine dinucleotide phosphate, reduced form; SDR, short chain dehydrogenase/reductase; Se, secretor; VWF, von Willebrand factor.

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